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METABOLIC STUDIES OF ENERGY DENSE COMPOUNDS FOR AEROSPACE NUTRITION

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The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

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Foreword

This investigation is a continuation of studies reported in WADD Technical Report 60-575 (August 1960) and in MRL-TDR-62-35 (May 1962). The research described in this report was carried out from 28 November 1962 to 15 September 1964 at the Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Cambridge 39, Massachusetts. The work was done under Contract AF 33(657)-7660, in support of Project 7164, "Biomedical Criteria for Aerospace Flight," and Task 716405, "Aerospace Nutrition," for the Biospecialties Branch, Physiology Division, Biomedical Laboratory, Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio. 1/Lt. John Vanderveen served as contract monitor. Personnel participating in the project were: Sanford A. Miller, PhD, Henry A. Dymsza, PhD, Steven R. Tannenbaum, and Samuel A. Goldblith, PhD.

This technical report has been reviewed and is approved.

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Abstract

The aim of these studies was the development of model compounds with which information useful in understanding energy metabolism might be obtained to aid in development of food for space travel. Seven-month studies feeding rats with 1,3-butanediol have been completed. The results of these studies confirm the utilization of this compound as an energy source. Measurement of a number of metabolic parameters at the completion of the study support the contention that 1,3-butanediol is probably metabolized through carbohydrate rather than fat pathways. Metabolism studies with 2,4-dimethylheptanoic acid labeled with C^{14} in the alpha methyl group indicate that this compound as predicted is oxidized through propionate. Design and construction details of the direct animal calorimeter are presented. Results of a limited number of studies with rats fed various diets indicate that the device fulfills its design functions.

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SECTION I.
Introduction

The relationship between diet and environment represents one of the most interesting areas of nutritional research. Modern thought in nutrition is that the environment can determine nutritional need. Conversely, the diet can modify the response of the organism to its environment. In the early years of planning for man's entrance into space, the problems of weight and volume were paramount. At that time, an essential requirement for this journey was a light, highly concentrated source of nutrients. Of the many approaches examined, one was concerned with the investigation of new and unusual nutrient sources. The principal goal in this study was the production of high energy diets in which all or a principal portion of the energy would be supplied by synthetic compounds having certain caloric densities greater than that of the carbohydrates and capable of being fed at greater levels than the fats.

These investigations were divided into three sections. The first was concerned with available compounds not normally associated with the diet. The diol, 1,3-butanediol, was selected as the model for the group. The second approach was concerned with the *design* and synthesis of new compounds capable of performing specific metabolic tasks. The fatty acid, 2,4-dimethylheptanoic acid, used in these studies, was designed to supply energy equivalent to that of a fatty acid without the usual deleterious effects associated with feeding large amounts of these materials. The third section of this program was concerned with the design and construction of a highly sensitive and rapid animal calorimeter for use in the study of energy metabolism.

The initial work in these studies was presented in earlier reports (refs. 1, 2). The present report is concerned with the continuation of these studies.

The aims of these studies were to develop *model* compounds with which information useful in understanding energy metabolism might be obtained. With this information, more useful and practical compounds may arise.

SECTION II.

Animal Experimentation

The objectives of the animal experimentation phase of this investigation were to establish a metabolic basis for the evaluation of potential compounds useful as synthetic dietary energy sources. In general, these studies have been concerned with the determination of the metabolic caloric density of these compounds as well as the investigation of the physiological and nutritional effects of feeding these materials.

Metabolic studies of two compounds are discussed. For the first, 1,3-butanediol, the final results of a long term growth and metabolic study are reported. In the case of the second compound, 2,4-dimethylheptanoic acid, data are reported of a study in which the compound was labeled with C^{14} in the alpha methyl group.

PROTEIN, FAT AND 1,3-BUTANEDIOL INTERRELATIONSHIPS IN HIGH ENERGY DIETS

In the previous report (ref. 2), preliminary data were reported of a 30-week study utilizing rats to determine the interactions and effects of adequate and high dietary levels of protein on the utilization of diets containing various levels of fat and 1,3-butanediol. At the conclusion of that study, samples of urine, blood, and liver were taken and used for the determination of various metabolic intermediates and products. In order to present as clear a discussion as possible, some of the data presented in the earlier report (ref. 2) are reported here. In addition a summary of pertinent literature concerning 1,3-butanediol also is included.

Commencing around 1949, reports on 1,3-butanediol began to appear in the literature. Fischer et al (ref. 3) reported that 1,3-butanediol had a low toxicity as indicated by an oral LD_{50} of 29.42 cm^3/kg in rats. Further studies by Meyer (ref. 4) in three generations of rats indicated that relatively low levels of 1,3-butanediol had no adverse effect on growth, fertility, and reproduction. Bornmann (refs. 5, 6, 7), in his three-part series of papers, also concluded that 1,3-butanediol was of low acute and chronic toxicity.

Specific nutritional use of polyhydric alcohols, including 1,3-butanediol, was reported by Schussel (refs. 8, 9). The polyols were fed to rats at levels of 5% to 40% of dietary calories. Of the 7 polyols tested, 1,3-butanediol was best tolerated. Low concentrations appeared to stimulate growth.

In our laboratory, data have been developed which demonstrate that 1,3-butanediol has a metabolic caloric density of approximately 6 Cal/g (ref. 1) and that it may possibly be metabolized through pathways resembling those of carbohydrate rather than fat (ref. 2).

In the present report, work representing a continuation of these studies is presented.

Experimental Procedures

Commercial grade 1,3-butanediol (BD) was used in all of the experiments. The compound met specifications as follows: boiling point, 207.5 C; specific gravity 20/20 C, 1.006; and a minimum purity of 99% by weight. While BD has unusual chemical stability, because of high hygroscopicity, care was taken to prevent water absorption in storage and handling.

The animals used in this study were Caesarean-derived "SPF" male rats (Charles River Laboratories) which weighed approximately 145 g each. More mature animals were used to avoid

the odor and palatability problems associated with the use of younger animals in the earlier studies (ref. 2). All animals were housed in individual wire bottom cages in temperature, humidity, and light controlled rooms.

The animals were arranged in fifteen groups of 10 rats each and were fed their respective diets for 30 weeks. Protein, fat and BD levels were the main experimental variables. In some diets, however, supplements of soy lecithin were used to try to increase fat absorption. Calcium lactate and sodium propionate were added to two of the diets to test for possible antiketogenic effects. The characteristics of the diet on a dry basis are given in table I. In addition, all of the diets contained 4% salt mix, 1.2% vitamin mix, and 4% agar by weight. Where supplied, carbohydrate was furnished by sucrose, dextrose and dextrin in the ratio of 2:1:1, respectively. The fat source consisted of 1 part corn oil to 3 parts lard. In order to facilitate the feeding of high levels of fat and BD, all diets were prepared as semisolid agar gels by incorporation of 1000 g of the dry diet with 750 ml of hot water containing the dissolved agar.

At the end of 30 weeks, the animals were placed in metabolism cages and fasted for 18 hours. Urine was collected in ice and was kept frozen in sealed vials until analysis could be performed. After removal from the metabolism cages, the animals were killed by decapitation, blood collected and livers and kidneys removed and weighed. Examination was made also for gross pathological changes. The blood was kept refrigerated, centrifuged, and the serum removed and frozen. An aliquot of liver was taken immediately upon dissection, weighed and placed in KOH. The remainder was frozen.

TABLE I

*Characteristics of diets used in 30-week long-term study**

	Diet No.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Protein, %	18	36	18	36	18	36	36	18	28	18	36	18	18	18	28
Fat, %	10	10	30	30	30	3	30	30	30	50	50	60	60	60	60
1,3-Butanediol, %	–	–	–	–	20	20	20	30	30	–	–	–	–	–	–
Carbohydrate, %	62	42	42	22	22	2	–	12	–	22	2	12	11	6	–
Lecithin, %	–	–	–	–	–	–	0.5	–	–	–	–	–	0.5	–	–
Calcium lactate, %	–	–	–	–	–	–	–	–	–	–	–	–	–	2.5	–
Sodium propionate, %	–	–	–	–	–	–	–	–	–	–	–	–	–	2.5	–

*Dry basis

Measurements were made of serum glucose (ref. 10), ketone bodies (ref. 11) and cholesterol (ref. 12). In the liver, determinations were made of glycogen (ref. 13) and phosphohexase isomerase (ref. 14). Urinary ketone bodies also were determined (ref. 11).

All of the data were statistically analyzed by the analysis of variance methods described by Snedecor (ref. 15).

Results

Data for the first 4 weeks of this long-term feeding study are given in table 2. Animals fed diets 8 and 9 which contained 30% BD consumed less food and gained less body weight than rats

fed any of the other diets. As a result, food and calorie efficiency were depressed by the 30% level of BD. The body weight gains of the 2 groups of rats receiving the 30% BD were significantly less ($P < 0.05$) than those of rats on any of the other diets. Within a specific fat level, weight gains were generally improved in the group receiving the higher protein level, but these differences were significant ($P < 0.05$) only in rats fed the 50% fat diets.

After 30 weeks of feeding (table III), animals fed BD (groups 5 to 9) were lighter in body weight than rats fed any of the other diets containing 30% or more fat (diets 3 and 4, 10 to 15).

TABLE II

Effects of protein, fat and 1,3-butanediol (BD) levels on 4-week weight gain, nutrient intake and nutrient efficiency

Group	Diet	No Rats	Weight Gain* g	Nutrient Intake (Dry basis) Food g	Protein g	Calories Cal	Nutrient Efficiency† Food %	Protein %	Calories %
1	10% fat + 18% protein	10	160 ± 6‡	448	81.0	1808	35.6	1.97	8.8
2	10% fat + 36% protein	10	174 ± 5	442	159.7	1783	39.3	1.09	9.7
3	30% fat + 18% protein	10	179 ± 8	399	72.1	2030	44.9	2.49	8.8
4	30% fat + 36% protein	10	173 ± 8	384	138.6	1952	45.1	1.25	8.9
5	30% fat + 18% protein + 20% BD	10	143 ± 8	361	65.3	1965	39.5	2.18	7.3
6	30% fat + 36% protein + 20% BD	9	159 ± 10	347	125.5	1888	45.8	1.27	8.4
7	30% fat + 36% protein + 20% BD + 0.5% lecithin	10	148 ± 10	320	115.8	1742	46.3	1.28	8.5
8	30% fat + 18% protein + 30% BD	10	109 ± 7	302	54.5	1746	33.1	1.83	5.7
9	30% fat + 30% protein + 30% BD	9	120 ± 11	308	85.3	1728	28.9	1.40	6.9
10	50% fat + 18% protein	10	143 ± 11	330	59.6	2027	43.4	2.40	7.1
11	50% fat + 36% protein	10	177 ± 5	343	123.8	2043	51.6	1.43	8.7
12	60% fat + 18% protein	10	164 ± 12	351	63.4	2341	46.8	2.59	7.0
13	60% fat + 18% protein + 0.5% lecithin	10	143 ± 7	313	56.5	2086	45.6	2.53	6.8
14	60% fat + 18% protein + 2.5% lactate + 2.5% sodium propionate	10	155 ± 11	320	57.9	2079	48.4	2.68	7.5
15	60% fat + 30% protein	10	176 ± 7	323	89.6	2098	54.3	1.96	8.4

*LSD at the 5% level = 25 g; at the 1% level = 35 g.

†Food efficiency = g body weight gain per 100 g food consumed. Protein efficiency = g body weight gain per 1 g protein consumed. Calorie efficiency = g body weight gain per 100 calories consumed.

‡Standard error.

TABLE III

Effects of protein, fat, and 1,3-butanediol (BD) on 30-week weight gain, nutrient intake, nutrient efficiency and survival

Group	Diet	No Rats	Weight Gain* g	Nutrient Intake (Dry basis) Food g	Protein g	Calories Cal	Nutrient Efficiency† Food %	Protein %	Calories %
1	10% fat + 18% protein	9	445 ± 20†	3381	609	13644	13.2	0.73	3.3
2	10% fat + 36% protein	10	448 ± 13	3284	1182	13253	13.6	0.38	3.4
3	30% fat + 18% protein	10	518 ± 35	3118	561	15863	16.6	0.92	3.3
4	30% fat + 36% protein	8	509 ± 40	3099	1116	15767	16.4	0.46	3.2
5	30% fat + 18% protein + 20% BD	9	491 ± 20	2913	524	15841	16.9	0.94	3.1
6	30% fat + 36% protein + 20% BD	9	447 ± 39	2867	1032	15589	15.6	0.43	2.9
7	30% fat + 36% protein + 20% BD + 0.5% lecithin	7	440 ± 34	2682	966	14586	16.4	0.46	3.0
8	30% fat + 18% protein + 30% BD	9	397 ± 37	2637	475	15269	15.1	0.84	2.6
9	30% fat + 30% protein + 30% BD	8	338 ± 60	2623	734	14726	12.9	0.46	2.3
10	50% fat + 18% protein	9	484 ± 40	2784	501	17098	17.4	0.97	2.8
11	50% fat + 36% protein	7	482 ± 18	2766	996	16500	17.4	0.48	2.9
12	60% fat + 18% protein	10	553 ± 42	2852	513	19011	19.4	1.08	2.9
13	60% fat + 18% protein + 0.5% lecithin	9	524 ± 29	2713	488	18084	19.3	1.07	2.9
14	60% fat + 18% protein + 2.5% lactate + 2.5% sodium propionate	7	432 ± 30	2680	482	17397	16.1	0.90	2.5
15	60% fat + 30% protein	8	564 ± 34	2718	761	17645	20.8	0.74	3.2

*LSD at the 5% level = 101 g, at the 1% level = 143 g
†Standard error

However, the weight gains of animals fed 20% BD (diets 5 and 6) were not significantly different from those of rats fed unsupplemented 30% fat diets (diets 4 and 5). As observed at 4 weeks, animals in groups 8 and 9 which received the 30% level of BD consumed the least food and had the lowest weight gains and food and calorie efficiencies. In almost all comparisons, the 2 groups of rats fed 30% BD had significantly lower ($P = 0.05$ to 0.01) weight gains than animals fed any other diet. Nevertheless, food and calorie efficiencies of animals fed the lower level of 20% BD (diets 5 and 6) were comparable to those of animals fed the 30% fat unsupplemented diets (diets 3 and 4).

No protein effect was apparent at 30 weeks. In addition, the lecithin, calcium lactate and sodium propionate supplements did not appear to be beneficial. Mortality was not excessive and did not appear to be related to the type of diet.

Liver and kidney weights, as recorded at the termination of the experiment after 30 weeks, are shown in table IV. There were no significant differences among any of the values when compared on a percentage of body weight basis.

The results of the determinations of metabolic intermediates and products are shown in fig. 1. Liver phosphohexase isomerase (PHI) generally decreased with increasing fat in the diet (fig. 1A).

TABLE IV

*Weights of livers and kidneys of animals fed diets containing various levels of protein, fat and 1,3-butanediol**

Group	Diet	Liver % Body weight	Kidney % Body weight
1	10% fat + 18% protein	2.05 ± 0.07†	0.55 ± 0.02
2	10% fat + 36% protein	2.19 ± 0.07	0.53 ± 0.02
3	30% fat + 18% protein	1.98 ± 0.07	0.47 ± 0.02
4	30% fat + 36% protein	2.10 ± 0.07	0.50 ± 0.02
5	30% fat + 18% protein + 20% BD	2.30 ± 0.06	0.52 ± 0.02
6	30% fat + 36% protein + 20% BD	2.20 ± 0.05	0.59 ± 0.03
7	30% fat + 36% protein + 20% BD + 0.5% lecithin	2.20 ± 0.02	0.56 ± 0.01
8	30% fat + 18% protein + 30% BD	2.40 ± 0.08	0.54 ± 0.01
9	30% fat + 30% protein + 30% BD	2.20 ± 0.14	0.63 ± 0.05
10	50% fat + 18% protein	2.10 ± 0.13	0.49 ± 0.02
11	50% fat + 36% protein	2.20 ± 0.10	0.48 ± 0.01
12	60% fat + 18% protein	2.03 ± 0.05	0.49 ± 0.02
13	60% fat + 18% protein + 0.5% lecithin	2.00 ± 0.04	0.48 ± 0.04
14	60% fat + 18% protein + 2.5% lecithin + 2.5% propionate	2.00 ± 0.09	0.57 ± 0.03
15	60% fat + 30% protein	2.10 ± 0.07	0.52 ± 0.02

*No significant differences exist among any of the liver or kidney values
†Indicates standard error

The exception to this pattern was a rise in activity at 50% dietary fat. When BD was added to the 30% fat diet, PHI activity increased with increasing dietary concentrations.

Serum glucose appeared to respond in a similar manner (fig. 1B) although the changes appear to be relatively small. In this case, however, there appeared to be a drop in serum glucose at 20% BD with an increase at 30% BD.

On the other hand, little or no changes could be observed in liver glycogen (fig. 1D) at dietary fat levels up to 50%. At 60% fat, however, there appeared to be a significant rise in liver glycogen. The addition of 20% BD to the diet had little effect on this parameter. A supplement of 30% BD, however, resulted in an increase in liver glycogen.

Changes in serum cholesterol are shown in fig. 1C. While there appeared to be a slight decrease in serum cholesterol with increasing dietary fat, the changes are not significant. The 1,3-butanediol, however, gives indications of being an effective hypocholesteremic agent, reducing serum levels from 150 mg% at 0% in the diet to 60 mg% when the diet was supplemented with 30% BD.

The levels of serum ketone bodies (SKB) are shown in fig. 1E and table V. SKB generally increased with increasing dietary fat up to 50%. When dietary fat was increased to 60% the level

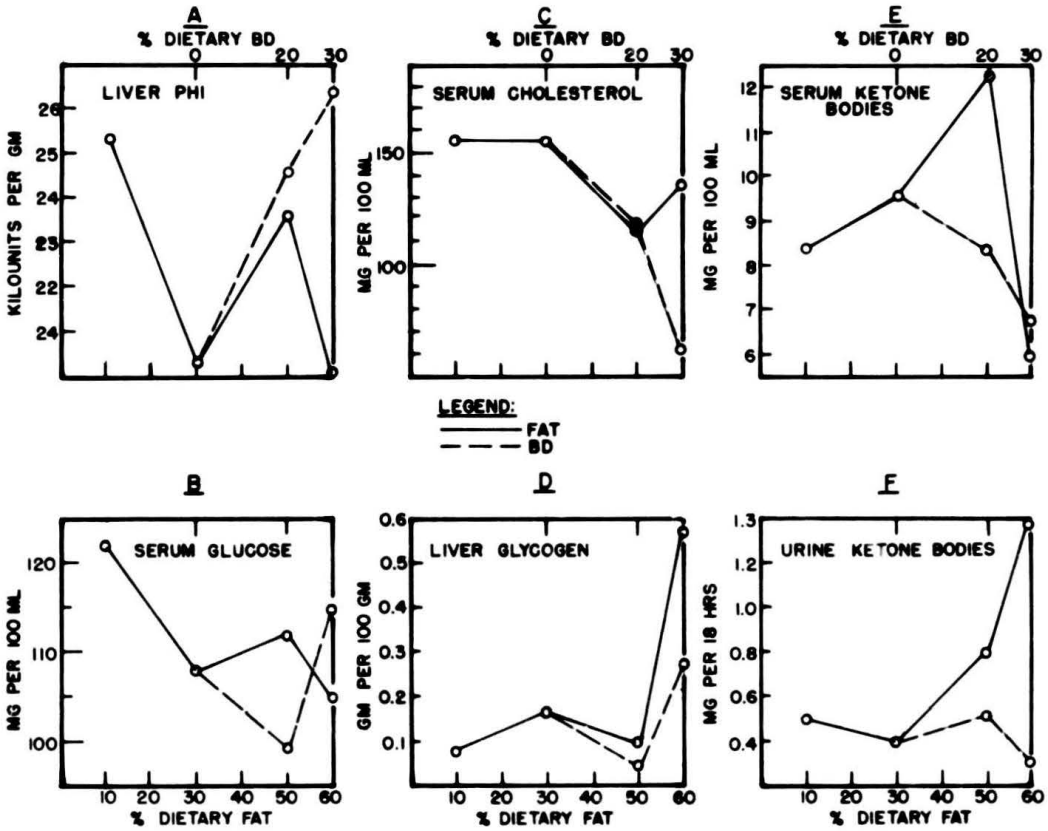


FIG. 1. Metabolic Parameters in Rats Fed Diets Containing Various Levels of Fat and 1,3-Butanediol

precipitously dropped, falling to 50% of the levels of 50% the animals fed fat diets. Similarly, additions of BD to the diet produced a slight decrease of SKB.

An inverse pattern can be observed when urinary ketone bodies (UKB) are considered (fig. 1F and table V). In this case, however, little if any change can be seen at dietary fat levels to 30%. At 50% dietary fat, however, UKB begin to rise reaching a maximum at 60% dietary fat. The addition of BD to the 30% fat diet, however, results in essentially no change in UKB. Also, urine volume appeared to increase with increasing levels of dietary fat (table V). The addition of BD to the diet appeared to slightly decrease urine volume.

Discussion

In this series of experiments, following adaptation, rats were able to utilize 20% BD as a carbohydrate replacement in 30% fat diets. While utilization of diets containing 30% BD was impaired as indicated by body weight gain and nutrient efficiencies, there was no unusual morbidity or mortality. The results reported are in agreement with Bornmann's (ref. 7) conclusions concerning the low order of toxicity of BD. They also support Schussel's (ref. 9) original viewpoint that BD can serve as a potential source of dietary energy.

TABLE V

Ketone Bodies in Serum and Urine at 30 Weeks

	10% Fat	30% Fat	50% Fat	60% Fat	30% Fat + 20% BD	30% Fat + 30% BD
Serum Ketone Bodies*						
mg/100 ml	8.37	9.60	12.33	5.97	8.48	6.84
Urine Volume						
ml/18 hours	6.85	8.20	11.40	13.56	8.17	5.55
Urine Ketone Bodies						
v/ml	74.00	48.40	70.70	95.40	63.90	63.00
Total Urine Ketone Bodies						
mg/18 hours	0.51	0.40	0.81	1.29	0.52	0.35
Urine Ketone Bodies						
mg/kg/18 hours	6.29	5.25	6.70	29.88	5.87	6.29

*As acetone

The initial attempt in our laboratory to feed high levels of BD to weanling rats (ref. 2) indicated that the poor performance in terms of weight gain but not of nutrient efficiency on 10% and 20% BD was due to a reduction in food intake. Further study of this problem by paired feeding (ref. 2) showed that animals pair-fed 5% and 20% BD for 3 weeks were similar or superior to unsupplemented control rats in weight gain and food and protein conversion efficiency. However, in the caloric tests and in the short term graded level *ad libitum* and paired feeding test the animals did not appear to be fully utilizing the BD. Some insight into this problem was gained by an isocaloric force-feeding study (ref. 2) which demonstrated the need for an adaptation period of at least 1 week for maximum utilization of BD.

At the conclusion of the first 4-week period of the long-term study presented in this report, there was a marked decrease in food intake, body weight gain, and food and calorie efficiency of animals fed diets of 8 and 9 which contained 30% BD. These adverse effects of feeding the high level of 30% BD continued throughout the entire 30-week feeding period.

The principle long-term effects of feeding BD are readily demonstrated if the results from groups fed similar levels of fat and BD are combined and compared. Fig. 2 shows such a combined group growth curve. The curve indicates that except for the last 5 weeks on test, weight gains of animals fed 20% BD were rather similar to those of animals fed the 10%, 50% and 60% fat diets. Weight gains of rats on 30% BD lagged considerably behind the others. The superior weight gains on the 30% fat diets appears to confirm the reports of Scheer et al (ref. 16) that 30% may be the optimum level of dietary fat for the rat. However, there is no agreement among workers over the proper level of dietary fat.

Examination of the combined 30-week data in table 3 indicates that weight gains and utilization of diets containing 20% BD were not significantly impaired. Food efficiency on these diets was 16.3 as compared to a value of 16.5 for the 30% fat unsupplemented diets. Similarly, average caloric efficiency was 3.0 on the 20% BD diets as compared to 3.3 for 30% fat unsupplemented diets and 2.9 for the 50% and 60% fat diets.

In the case of the 30% BD diets, we must conclude from a consideration of all parameters measured that, at this level of feeding, there was a significant impairment in diet utilization. Since Hess and Kopf have reported (ref. 17) that BD retards the absorption of drugs, absorption

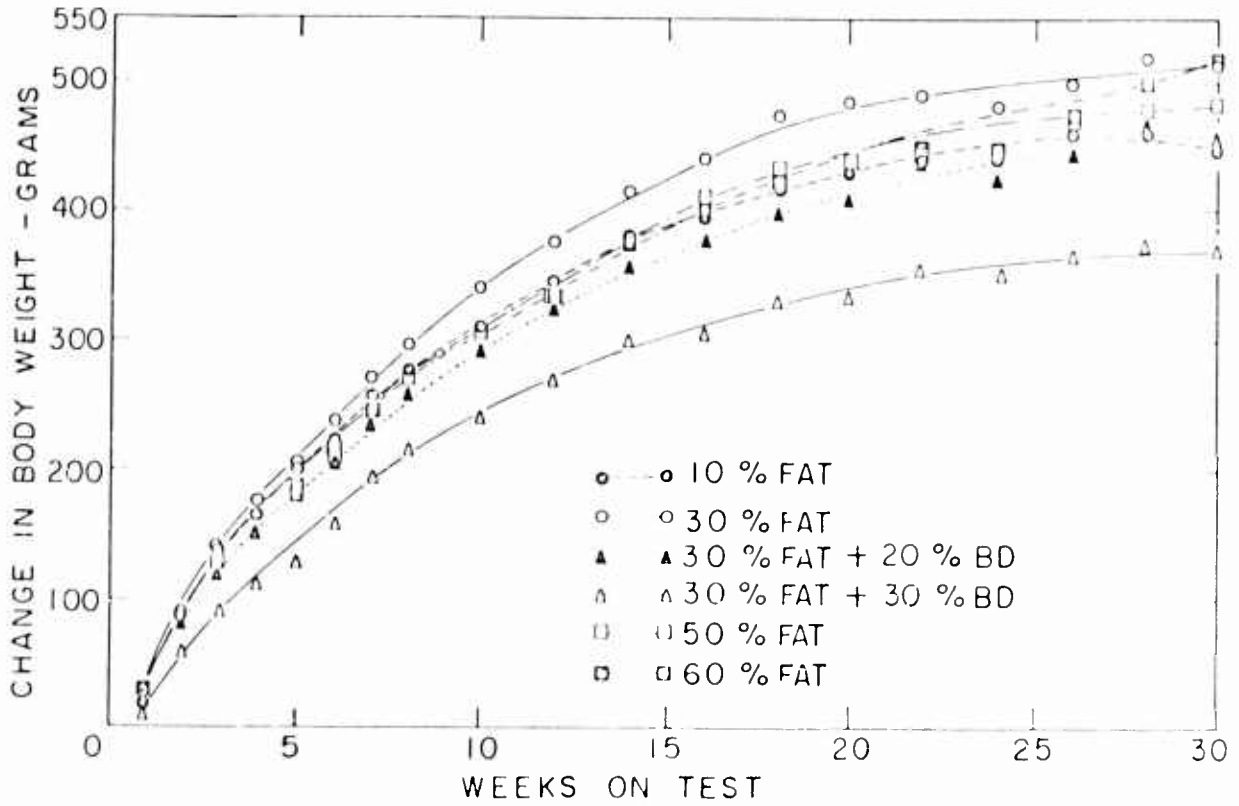


FIG. 2. Weight Gains of Rats Fed Diets Containing Various Levels of Fat and 1,3-Butanediol

may be one of the factors responsible for the lower utilization of diets containing high levels of BD.

Comparison of the 30% fat diets, with and without 20% added BD, reveals that animals fed the BD-containing diet consumed 9% less food in 30 weeks. On the other hand, the highest 30-week food conversion (18.9%) was obtained in animals fed the 60% fat diets. Furthermore, the combined data (table III) indicate that the 30-week average weight gains of rats, with the exception of those fed 30% BD, were not statistically different on levels of 10%, 30%, 50% and 60% fat. While Michelsen et al (ref. 18) used a diet containing 63% fat to produce obesity, in this experiment there were as many obese rats on 30% fat as on 60% fat. Unlike the observations of Yoshida et al (ref. 19) the caloric consumption of rats generally increased as the level of fat in the diet was raised from 10% to 30%, 50% and 60%.

Somewhat similar to the results reported by French et al (ref. 20), after 4 weeks on test, the levels of protein were apparently without effect on the superior food utilization obtained with the high fat diets.

We recognize that, while carbohydrate may modify the response obtained with a diet, there may not be a specific requirement for carbohydrate (ref. 21). In this experiment, there did not appear to be any detrimental effects from feeding essentially no carbohydrate, except the small amount used as a diluent in the vitamin mix, in one of the 60% fat diets (diet 15) for 30 weeks.

Also, the feeding of 2% or less carbohydrate in diets 6, 7, 9 and 11, while difficult to evaluate, appeared to be without any gross harmful effect in terms of weight gain and nutrient efficiency.

Williams (ref. 22) reported that BD is probably metabolized to beta hydroxybutyric acid and then excreted via the kidneys. The results of this study did not appear to support this contention. Since no significant increase in serum or urine ketone levels was observed when BD was fed, BD apparently is not metabolized via the ketone bodies.

Further support for this concept can be obtained from the results of the other assays. If BD had been metabolized through lipid pathways, a decrease in liver phosphohexase isomerase (PHI) might be expected as occurred when increasing amounts of fat were added to the diet. In addition, serum glucose and liver glycogen would be expected to respond as when fat was added to the diet. Since the changes, with the exception of liver glycogen, were opposite to those observed with increasing dietary fat, BD is probably metabolized through some portion of carbohydrate metabolic pathways rather than through those of fat metabolism. Within this context, the possible increases in liver glycogen with increasing dietary BD may be explained on the basis that the feeding of BD results in a net synthesis of glycogen.

The marked decrease in serum cholesterol with increasing dietary BD cannot be explained at this point. Whether this represents an inhibition of liver cholesterol synthesis or an interference with serum transport is not known.

A final comment should be made of the anomalous behavior of the animals fed the diets containing 50% fat. In the assays for PHI, glucose and glycogen, these animals consistently demonstrated a response opposite to what might be expected. Although these responses probably represent an artifact of the experiment, they are being reexamined to determine their validity.

There is no question, therefore, that the feeding of BD at levels of up to 20% of the diet (at least) results in no gross deleterious effect in terms or nutrient utilization and weight gain. Furthermore, the feeding of BD can apparently supply at least 6 kcal/g resulting in a net increase in caloric density of the normal ration. In addition, there apparently is no increase in serum and urinary ketone body levels when BD is fed, thus demonstrating an increased usefulness for this compound as compared to fat. Metabolically, BD is apparently not metabolized through lipid pathways, since its effect on PHI and serum glucose resembles those of carbohydrate rather than fat.

THE METABOLISM IN VIVO OF 2,4-DIMETHYLHEPTANOIC ACID

Introduction

In a previous report (ref. 1) an argument was presented which demonstrated that most physiologically practical diets of high caloric density would consist of 75% or more of fat. Prior to the incorporation of large amounts of fat into a ration, either the accumulation of ketone bodies must be prevented or that the rate of acetate production must be controlled or that fatty acids be fed which are not metabolized to acetate. It was further postulated that it may be possible to design a fatty acid that, upon beta oxidation, would be metabolized to propionate rather than acetate. This, in effect, would avoid the difficulties encountered in high fat diets by restricting the production of ketone bodies.

To initiate the present study, it was decided to synthesize a multimethylated fatty acid, 2,4-dimethylheptanoic acid (DMHA), and study its metabolism to determine if this hypothesis is correct.

The synthesis and initial toxicity studies of this compound have been previously reported (ref. 2). The present report is concerned with a study of the metabolism of 2,4-dimethylheptanoic acid labeled with C^{14} in the alpha methyl group. For purposes of control and comparison, propionate-3-C^{14} was also studied.

Experimental Procedures

Young male Sprague-Dawley derived (Charles River Breeding Laboratory) rats were used in these studies. Prior to the time of the experiment, the animals were housed in individual, wire bottomed cages, under conditions of controlled light, temperature, and humidity.

The test compounds (DMHA and propionate) were incorporated into agar gel diets. The composition of the diet is given in table VI. The dry ingredients (with the exception of the vitamin mix) were weighed and premixed by hand. The test compound was dissolved in the corn oil and added to the dry mix. The agar was melted in boiling water and then added to the other ingredients while mixing rapidly. Mixing was continued until the diet had cooled sufficiently for the addition of the vitamin mix and choline chloride. The mixture was then poured into shallow pans to set.

Animals were offered the test diet containing the non-radioactive test compound ad libitum for an initial adaptation period of approximately 3 days. Following this period, the animals were fed by offering the diet for 2 hours each morning until they had been trained to consume their daily ration within this period. The training period normally took from 2 to 3 weeks. At the end of this time, the average daily food consumption was from 25 to 30 g.

TABLE VI

Diet

	Grams
casein	22.0
sucrose	12.2
dextrose	24.6
dextrin	12.3
salt mix W*	4.0
vitamin mix†	1.0
choline Cl (50% solution)	0.4
agar	3.5
corn oil	19.8
test compound (DMHA‡ or propionate§)	0.2
water	100

*L. G. Wesson, Science 75 339 (1932).

†Vitamin mix supplies the following per 100 grams dry diet: vitamin A, 300 I.U.; vitamin D, 30 I.U.; vitamin E, 2.5 I.U.; vitamin K, 0.5 mg.; thiamine HCl, 1 mg.; riboflavin, 2 mg.; niacin, 5 mg.; ascorbic acid, 20 mg.; pyridoxine, 1 mg.; para amino benzoic acid, 10 mg.; calcium pantothenate, 5 mg.; folic acid, 0.2 mg.; inositol, 20 mg.; biotin, 0.05 mg.; vitamin B_{12}, 0.005 mg.

‡DMHA = 2,4-dimethylheptanoic acid.

§Sodium propionate-3-C^{14} was purchased from New England Nuclear Corporation, Boston, Mass.

Animals were selected by weight for the metabolism studies. All animals were weighed the morning of the experiment prior to feeding. The animals selected for the test were placed in all-glass metabolism cages suitable for the collection of respiratory carbon dioxide and the separation of urine and feces. After an adaptation period of 1 hour, the animals were fed 20 g of diet containing the radioactive test compound. General experimental details are given in table VII. The animals tested for 48 hours were re-fed 20 g of non-radioactive test diet at 24 hours. The specific

activity of the propionate was 144,000 disintegrations per min./mg (dpm/mg) calculated as propionic acid. The specific activity of the DHMA was 50,000 dpm/mg.

Respiratory carbon dioxide was collected as follows. Dry, carbon dioxide free air was pulled through the individual cages into a solution of ethanolamine-methylcellosolve (1:2) (ref. 23). The air was redried over anhydrous calcium sulfate before passage into the ethanolamine solution. The collection efficiency of carbon dioxide of this procedure has been calculated to be 99.5%.[1] The total volume of ethanolamine solution used for a collection period was 10 ml for 1 hour or less, and 7.5 ml per hour for collection periods greater than 1 hour.

[1] Q. Rogers, MIT, personal communication, 1962.

TABLE VII

General Experimental Detail

Animal No.*	Weight, gms.	Compound Tested	Duration of Experiment, hrs.	Spilled Food, %
1-12P	190	propionate	12	4.00
2-12P	192	"	12	6.75
4-12P	196	"	12	0
5-48P	180	"	48	0
10-48P	183	"	48	0
5-12D	207	DMHA†	12	0
7-12D	225	"	12	12.82
10-12D	223	"	12	2.45
4-48D	198	"	48	0
6-48D	198	"	48	6.55

*In the code employed in these studies, the first number refers to the individual animal, while the second number refers to the duration of the experiment. The letter refers to the compound tested.

†DMHA = 2,4-dimethylheptanoic acid.

The ethanolamine solutions were refrigerated immediately after use in the collection of carbon dioxide. Since these solutions darken very rapidly upon standing, aliquots were taken as soon as possible for determination of radioactivity. Radioactivity in the carbon dioxide was determined in a fully automatic Tri-Carb Liquid Scintillation Counter using High Voltage tap 7 (1040 V), and discriminator settings of 10 and 100 V. A 3-ml aliquot of the ethanolamine solution was pipetted into 20 ml low-potassium glass vials, 15 ml of scintillator solution[2] was then added. The solutions were mixed well by hand and placed in the freezer at -4°C for at least 30 minutes before counting. All samples were counted to at least 10,000 counts and the counting efficiency determined by recounting with an added internal standard (toluene-C^{14}, New England Nuclear Corp., Boston, Mass.).

Feces and spilled food were separated by hand with a pair of forceps. This is feasible for agar gel diets except when the animal crumbles the diet with its paws. With this technique, the amount of spilled food can be determined with an accuracy of greater than 90%. However, the small amount of activity found in the feces is greatly affected by traces of spilled food or by contamination with urine.

[2] Toluene-methylcellosolve (2:1, containing 0.55% PPO (2,5-diphenyloxazole).

The feces and spilled food were hydrolyzed with hydrochloric acid on a steam bath for 2 hours, neutralized with saturated sodium carbonate to phenolphthalein endpoint, then centrifuged at 2000 rpm for 5 minutes. The insoluble material was washed with alcohol and water, and the washings added to the supernatant. The supernatant and washings were then made up to volume with alcohol in a 50-ml volumetric flask. A 2-ml aliquot was taken for counting, and 15 ml of scintillator solution (ref. 24) was added. If necessary, the sample was decolorized prior to the addition of scintillator solution as follows: to the 2-ml aliquot in the counting vial was added 0.2 ml of 30% hydrogen peroxide, and the solution heated in the tightly closed vial at 80 C for 6 hours.

At the end of the 48-hour experiment the animals were killed by decapitation and the blood collected. The stomach and the entire intestinal tract were excised and their contents washed out with distilled water. In the case of the 12-hour experiment the stomach and intestinal contents were washed into separate vessels. The radioactivity in these samples was determined in a manner identical to that employed for feces and spilled food.

The urine was collected at the end of the experiment. After heating in a boiling water bath for 30 minutes, and centrifuging to remove precipitated protein, the urine was made up to a convenient volume. A 2-ml aliquot was then decolorized with hydrogen peroxide and counted as previously described for feces and spilled food.

The urine collected from animal number 5-12D was concentrated to a small volume in a rotary evaporator under reduced pressure, acidified with sulfuric acid to pH 1 and continuously extracted with ethyl ether for 18 hours. The ether extract was dried over anhydrous sodium sulfate. It was then concentrated to a volume of 10 ml with a stream of dry nitrogen. The radioactivity in a 1-ml aliquot was determined by counting with 10 ml of scintillator solution.[3] The remaining 9 ml were concentrated to a volume of 1 to 2 ml and esterified with diazomethane.

The urine of animals 7-12D and 10-12D were combined and reduced to a small volume in a rotary evaporator under reduced pressure. Ten ml of 10% potassium hydroxide were added and the resulting solution extracted with petroleum ether to remove free neutral lipid. The aqueous, basic solution was then heated on a steam bath for 1 hour, brought just to dryness in a rotary evaporator, acidified to pH 1 and then continuously extracted with ether for 18 hours. The ether extract was then treated as described above for the determination of radioactivity and esterification of acidic material.

The urine of animals 4-48D and 6-48D were prepared in the same manner as the urines of animals 7-12D and 10-12D, with the exception that the ether extraction was performed in a separatory funnel (batch extraction).

A synthetic mixture of carboxylic acids was studied in order to test the efficacy of the method to esterify and separate by gas chromatography the types of carboxylic acids expected in urine. The esters that have been studied are given in table VIII. Fumaric acid and α-keto acids were not esterified by this procedure. Gas chromatographic separation of all of the esters listed in table VIII has been achieved on two different columns. A 1-meter column containing 10% (w/w) Carbowax 4000 on Johns-Manville Chromosorb P, 60/80 mesh, at 150° C brought about the separations shown in table IX. All the compounds studied with the exception of dimethyl glutarate and dimethyl 2-methylglutarate were separated under these conditions.

Improved resolutions and specifically improved separation of the lower boiling esters was achieved by using a 1-meter, 10% diethyleneglycol succinate (DEGS) on 60/65 mesh, acid washed Chromosorp P column at 100°C (table X). Resolution of higher boiling compounds than those

[3] Toluene containing 0.04% POPOP and 0.4% PPO

shown in table X could be achieved on the DEGS column at higher temperatures than 100°C. In general, the resolution of a group of compounds in a particular boiling range was better achieved on the DEGS column than on the Carbowax column.

TABLE VIII

Compounds Studied During Development of a Gas Chromatographic Method for Analysis of Methyl Esters of Certain Mono- and Polycarboxylic Acids

Monocarboxylic Esters	Dicarboxylic Esters		Tricarboxylic Esters
Methyl 3-hydroxybutyrate	Dimethyl oxalate	Dimethyl 2-metylmalonate	Trimethyl citrate
Methyl 4-methylheptanoate	Dimethyl malonate	Dimethyl 2-methylsuccinate	
Methyl 2,4-dimethyl-heptanoate	Dimethyl glutarate	Dimethyl 2-methylglutarate	
	Dimethyl adipate	Dimethyl malate	
	Dimethyl sebacate		

TABLE IX

Gas Chromatographic Separation of Certain Methyl Esters on the Carbowax Column at 150°C

Ester	Relative Retention Time*
Methyl 2,4-dimethylheptanoate	0.378
Dimethyl oxalate	0.440
Dimethyl 2-methylmalonate	0.475
Dimethyl malonate	0.598
Dimethyl 2-methylsuccinate	0.890
Dimethyl succinate	1.00
Dimethyl glutarate	1.49
Dimethyl 2-methylglutarate	1.49
Dimethyl adipate	2.48
Dimethyl 2-hydroxysuccinate	5.15
Dimethyl sebacate	14.4
Trimethyl citrate	24.8

*Relative to methyl succinate (t_r = 4.1 min.)

The esterfied urine extracts were chromatographed on the DEGS column at 100°C. Under these conditions the latest peak emerged at about 75 minutes. The entire effluent of the chromatograph was trapped directly in the counting vials in toluene-PPO-POPOP scintillator. Individual peaks, or, where this was not possible, groups of peaks, were also trapped in this manner for determination of radioactivity.

When individual peaks on the DEGS column contained significant amounts of radioactivity, they were trapped in 1-ml conical centrifuge tubes at liquid nitrogen temperature. This trapping process was repeated until sufficient material had been collected for infrared analysis.

TABLE X

Gas Chromatographic Separation of Certain Methyl Esters on the DEGS Colums at 100°C

Ester	Relative Retention Time*
Dimethyl oxalate	0.383
Dimethyl 2-methylmalonate	0.461
Dimethyl malonate	0.651
Dimethyl 2-methylsuccinate	0.804
Dimethyl succinate	1 00
Dimethyl 2-methylglutarate	1.49
Dimethyl glutarate	1.68
Dimethyl adipate	2.75

*Relative to methyl succinate ($t_r = 22.4$ min.)

Immediately following decapitation, approximately 500 mg of liver was removed from the animal, weighed as quickly as possible, and then immersed in 5 ml of 30% (w/v) potassium hydroxide. Glycogen was then isolated by the method of Good, Kramer, and Somogyi (ref. 13). The isolated glycogen was converted to glucose by hydrolysis with 0.6 N hydrochloric acid in a boiling water bath for 2 hours. Glucose was then determined colorimetrically in the Technicon Autoanalyzer by measuring the extent to which ferricyanide was reduced to ferrocyanide. This method gave a linear relationship between optical density and glucose over the range 50 to 2000 δ of glucose. Radioactivity in glucose was determined by counting a 1-ml aliquot of the glycogen hydrolyzate in 15 ml of scintillator solution[4] at High Voltage Tap 5 (960 V).

The washings and supernatants collected during the isloation of glycogen were combined and extracted with petroleum ether (bp 30-60°C) to isolate nonsaponifiable material. The petroleum ether was evaporated with a stream of dry nitrogen. The residue was then redissolved with 2 ml of petroleum ether, transferred to a counting vial, and 10 ml of scintillator solution[5] added. Counting was done at High Voltage Tap 4 (880 V).

The extracted alcohol-aqueous phase was acidified with concentrated hydrochloric acid to pH 1 and re-extracted with chloroform-heptane (2:1, bottom phase) to remove fatty acids. The extracted fatty acids were taken to dryness overnight in a vacuum dessicator. The residue was redissolved in petroleum ether and counted as described above for nonsaponifiable material.

The upper aqueous alcohol phase was adjusted to a convenient volume, and a 2-ml aliquot counted under the conditions described for feces and spilled food.

Following the removal of a section of liver, the left epididymal fat pad was removed, weighed, and placed in 10 ml of chloroform-methanol (2:1). The fat pad was allowed to remain in the chloroform-methanol, with occasional agitation, for 24 to 48 hours. The solvent was then decanted, and the process repeated twice with fresh 5-ml portions of solvent. The combined extracts were brought to dryness with a stream of nitrogen, and then counted as described for liver fatty acids.

The remaining carcass, including the washed stomach and intestines, and blood, were stored at −40°C until analysis. The carcass was then freeze-dried in the MIT Pilot Plant Freeze Drier. The dried carcass was weighed and then dissolved in 2 liters of 15% (w/v) potassium hydroxide

[4] Xylene:dioxane:cellosolve = 1:3:3, containing 1% PPO, 0.5% POPOP, and 8.0% naphthalene.
[5] Toulene containing 0.04% POPOP and 0.4% PPO.

in 40% (v/v) ethyl alcohol. A 25-ml aliquot of the carcass solution was acidified to pH 1 with concentrated hydrochloric acid, and extracted with chloroform-heptane (2:1) to remove fatty acids. The activity in the fatty acids was determined as described above for liver fatty acids. A 2-ml aliquot of the remaining aqueous phase was decolorized with hydrogen peroxide and counted as described for feces and spilled food.

Results

The cumulative percent recovery in respiratory CO_2 of C^{14} activity ingested from propionate and DMHA is shown in figure 3. Since the number of animals in each group was relatively small, the entire range of values is plotted at each point rather than the mean and standard error.

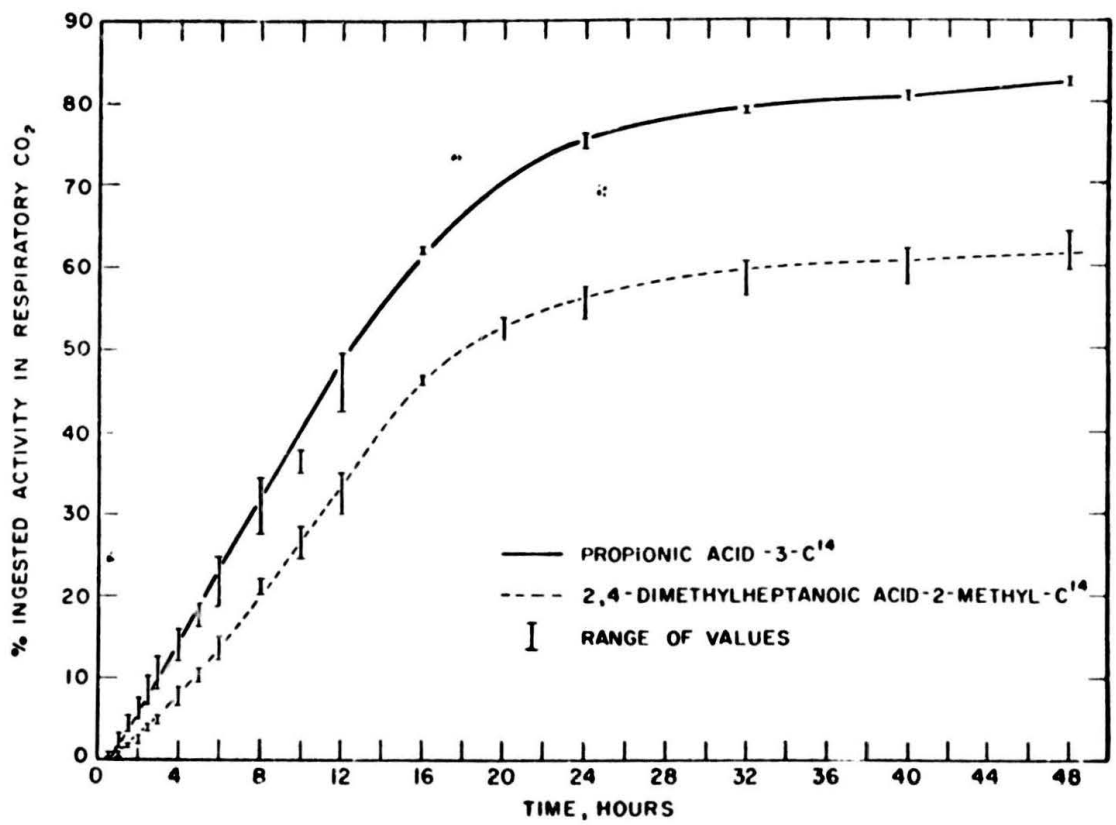


FIG. 3. Cumulative Percentage of Recovery of Ingested Activity in Respiratory CO_2

As illustrated in figure 3, the time required for 50% conversion of DMHA to CO_2 is 6 hours longer than that required for propionate conversion. At 48 hours 20% more propionate than DMHA had been converted to CO_2.

The rate of appearance in respiratory CO_2 of ingested activity from propionate and DMHA is shown in figure 4. These data were calculated by dividing the total activity accumulated in a given collection period by the number of hours in that collection period. The values thus obtained were plotted at the time corresponding to the middle of that collection period. In figure 4,

the rate of oxidation of DMHA to CO_2 is initially slower than that of propionate. The rapid decrease in rate of oxidation of propionate at approximately 6 hours occurred in each animal tested. DMHA also showed a decrease in rate of oxidation to CO_2 at 10 to 11 hours. The overall

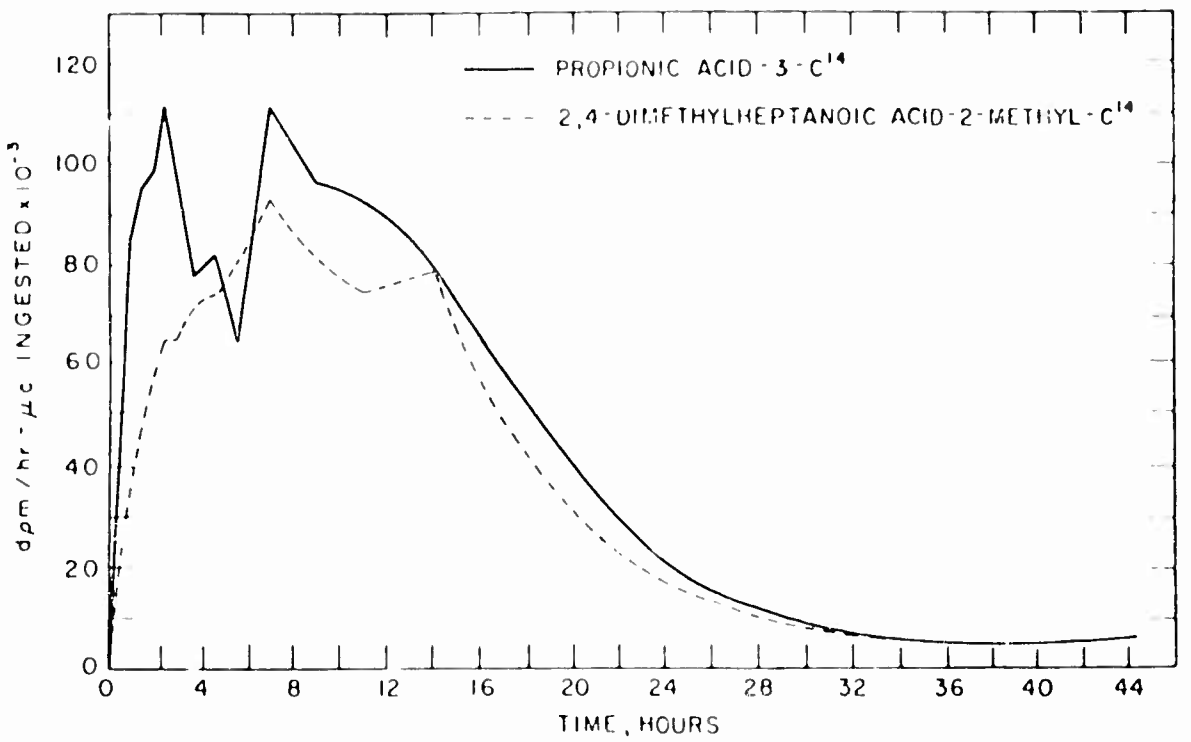


FIG. 4 Rate of Appearance of Ingested Activity in Respiratory CO_2

TABLE XI

Summary of the Recovery of Radioactivity from Propionic Acid-3-C[14]

	Animal No. 1-12P	2-12P	4-12P	5-48P	10-48P
Stomach contents	12.79	12.17	9.77	— — —	— — —
Intestinal contents	0.16	0.32	0.40	0.29	0.21
Respiratory CO_2	42.90	45.25	45.90	82.07	83.03
Urine	2.30	2.56	2.42	2.50	2.51
Feces*	1.25	1.61	0.015	0.34	0.17
Total Carcass Lipid	5.97	5.16	4.29	4.42	5.29
Total Carcass Nonlipid	26.85	23.25	22.24	15.35	15.63
Tissues removed for analysis	1.02	1.34	0.85	0.24	0.26
Recovery % of administered radioactivity	93.24	91.66	85.89	105.21	107.10

*Feces data valid only for total recovery due to contamination by urine and spilled food.
†Values are given in percent of ingested activity.

TABLE XII

Summary of the Recovery of Radioactivity from 2,4-Dimethylheptanoic acid-2-methyl-C^{14} *

	Animal No. 5-12D	7-12D	10-12D	4-48D	6-48D
Stomach contents	25.29	5.70	8.83	---	---
Intestinal contents	4.97	6.71	5.74	0.21	0.32
Respiratory CO_2	30.00	34.17	31.59	63.99	59.59
Urine	11.52	1[illegible]	14.26	21.58	24.23
Feces	0	0.05	0.075	1.60	1.01
Total Carcass Lipid	10.86	14.21	16.30	7.10	6.57
Total Carcass Nonlipid	19.86	21.35	16.61	15.10	13.56
Recovery % of administered radioactivity	102.50	99.21	93.41	109.58	105.28

*Values are given in percent of ingested activity.

drop in rate, however, was much less, and the rate of drop slower than that obtained for propionate.

The amount of food spilled by each animal is reported in table VII as percent of the amount fed. The amount spilled food is generally small but quite variable.

The data for feces, stomach contents, and intestinal contents are given in tables XI and XII. In the experiment with propionate, the feces were contaminated with urine and spilled food. These data, therefore, are valid only for calculations of total recovery. Recovery from intestinal contents indicates that propionate was much more rapidly absorbed than DMHA. This may be due to the relative water solubilities of these compounds. Greater variability was observed for DMHA than for propionate in the amount of activity remaining in the stomach 12 hours after feeding. The rate at which food is consumed can significantly affect stomach emptying time.

All of the results reported in this investigation have been corrected for spilled food and are reported as percent of ingested activity.

The fraction of ingested activity that appeared in glycogen, fatty acids, and aqueous soluble material of liver tissue is shown in figure 5. An insignificant amount of radioactivity was found in the non-saponifiable fraction of liver tissue. At 48 hours, there appears to be little significant difference in any of these tissue components between propionate and DMHA. Although activity in liver fatty acids at 12 hours is slightly greater for DMHA than for propionate, it is not known how much of this may be due to unmetabolized DMHA. Furthermore, the overall incorporation of activity into fatty acids is quite small for both propionate and DMHA.

Acid-2-Methyl-C^{14} in Lipid-Soluble and Non-Lipid Soluble Fractions of Rat

The percent liver glycogen and the ratio of the specific activity of liver glycogen to the specific activity of the test compound is given in table XIII. The magnitude of this latter ratio is similar for both DMHA and propionate at both 12 and 48 hours. There is probably no statistical difference between propionate and DMHA in this respect. Although, at 12 hours, the amount of liver glycogen is greater for propionate than for DMHA, all of the values obtained are within the normal range for young adult rats.

A significantly greater amount of activity was found in the urine of animals fed DMHA than of those fed propionate (tables XI and XII).

The nature of the activity contained in the urine of animals fed DMHA, as percent of total activity in urine, is summarized as follows:

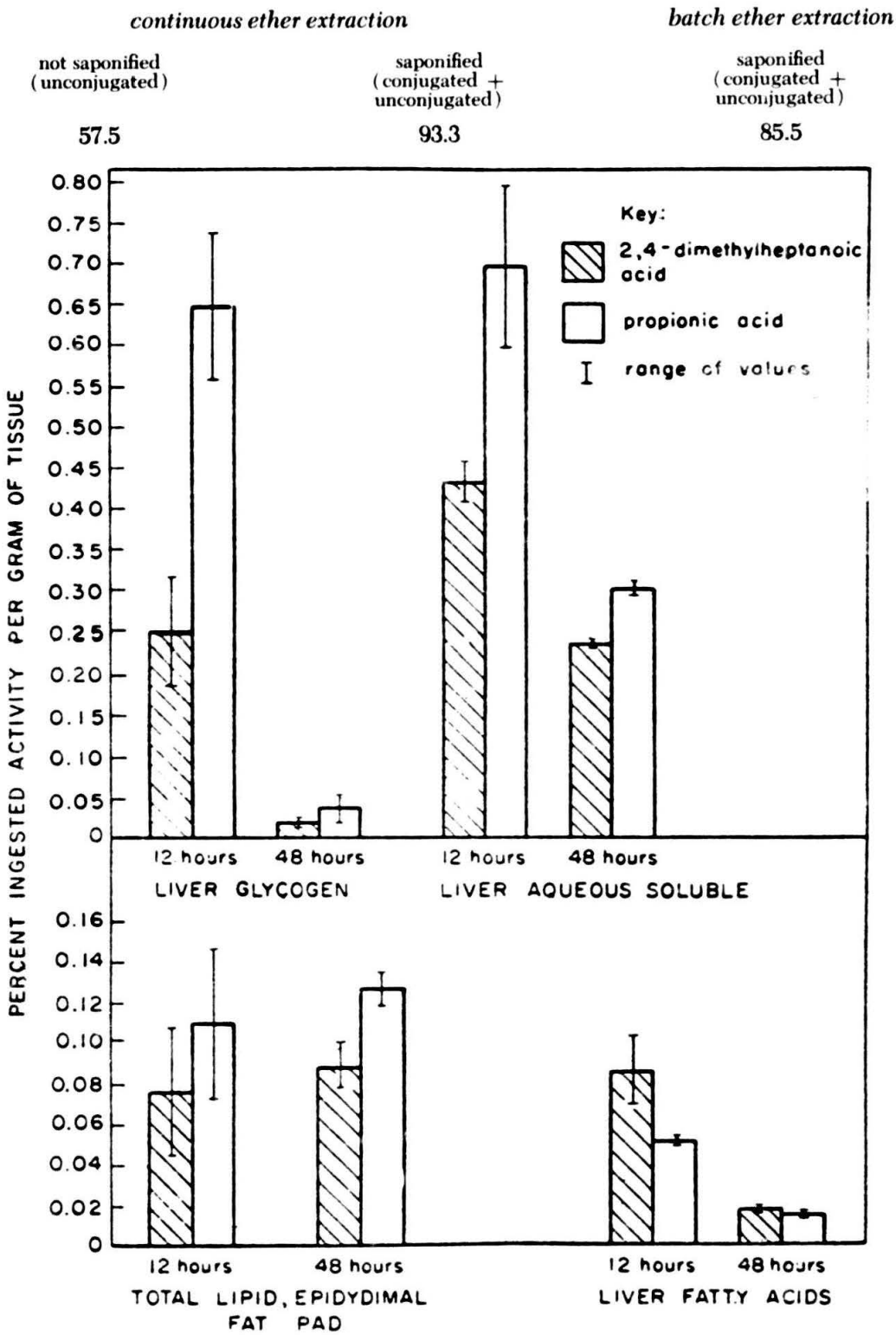


FIG. 5. Percent Ingested Activity from Propionate-3-C^{14} and 2,4-Dimethylheptanoic

Gas chromatographic analysis of the esterified urine extracts indicated that the peaks in the conjugated and nonconjugated fractions were similar, but not identical. Three peaks were of much greater intensity in the extract of saponified urine than in that of nonsaponified urine. This difference in intensity accounted for most of the difference in activity recovered by ether extraction.

TABLE XIII

*Radioactivity in Glycogen from Propionate and DMHA**

Animal No.	Compound Tested	Liver glycogen, %	$\frac{\text{sp. act. glycogen}}{\text{sp. act. compound}} \times 10^2$
1-12P†	Propionate	5.95	0.945
2-12P	"	5.45	0.715
4-12P	"	5.82	0.777
5-48P	"	2.52	0.165
10-48P	"	1.85	0.067
5-12D	DMHA*	4.04	1.290
7-12D	"	3.08	1.060
10-12D	"	3.42	0.664
4-48D	"	2.36	0.143
6-48D	"	2.17	0.080

*DMHA = 2,4 dimethylheptanoic acid

†The 12 or 48 in the Animal Number refers to the duration of the experiment in hours.

When compared to the retention volumes of other dicarboxylic esters on a DEGS column, the retention volumes of two of these peaks (A and B, figure 6) indicated that these fractions were in the boiling range of a C_7 dicarboxylic dimethyl ester. A and B accounted for 57.2% of total ether extractable activity. The third peak (C, figure 6) gave a retention time which did not correspond to any dicarboxylic ester, and contained 7.4% of the total ether extractable activity. The peak corresponding to dimethyl succinate contained 2.3% of the total ether extractable activity. These peaks were the only ones directly investigated, because they represented the major portion of urine activity, and were present in sufficient quantity to enable further investigation.

No peaks could be recognized which correspond to the diastereoisomers of DMHA. However, the early part of the chromatogram was quite crowded due to the presence of the many low boiling esters present in esterified urine extracts. A batch ether extraction, which was performed on the combined urine of animals 4-48D and 6-48D, would yield an extract which would favor the more lipid soluble material in urine. Succinic acid, for example, can only be completely isolated with 18 hours of continuous ether extraction. This batch extraction greatly reduced the intensity and number of the peaks in the early part of the chromatogram (eg, lactate, oxalate, glycolate, etc), while no effect could be observed on peaks A, B, and C. Significantly, although DMHA is known to be very readily extracted with ether, no peak for the methyl ester of DMHA could be observed in the esterified batch extract.

The infrared spectra of peaks A and B were essentially identical, both as pure liquids and in the carbon tetrachloride solution. This evidence, and the fact that no peaks for the diastereoisomers of DMHA were detected indicated that peaks A and B might be diastereoisomers. The spectra of A and B showed conclusively that these compounds were esters. The intensity of the

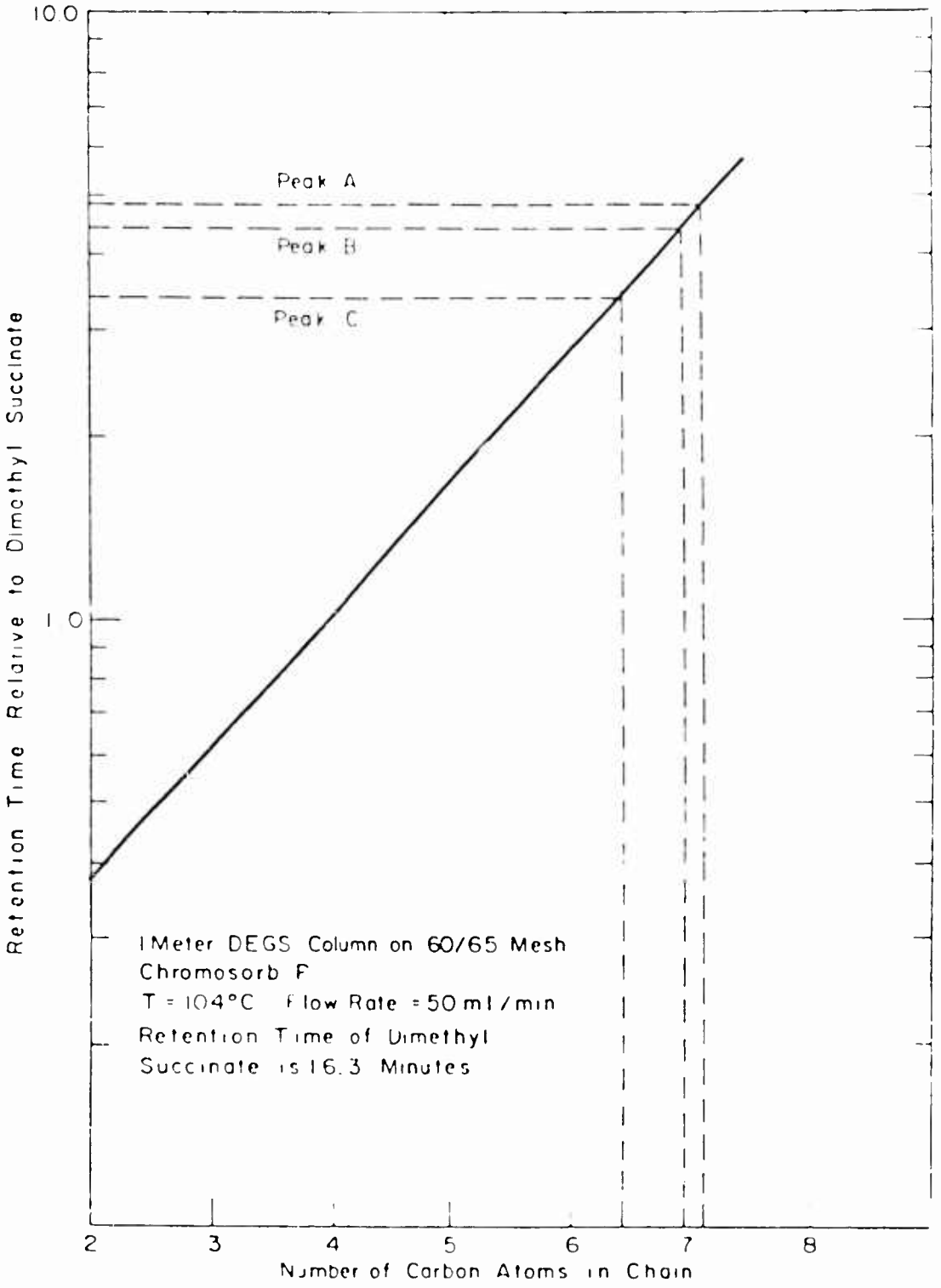


Fig. 6. Comparison of Retention Times of Certain Urinary Components with Known Dicarboxylic Esters

carbonyl band at 5.75 microns to the C-H band at 3.35 microns was similar to that found in dimethyl adipate and suggested that these compounds were dicorboxylic esters. The presence of a band at 7.25 microns indicated the presence of methyl groups in peaks A and B. Furthermore, the band intensities at 6.85 microns for C-CH_3 and at 6.96 microns for O-CH_3 were equal, indicating that the same number of methyl groups and methoxy groups might be present in each compound. On the basis of the above evidence and review of spectra of known esters, A and B were tentatively identified as diastereoisomers of dimethyl 2,4-dimethylpimelate, eg, Formula 1 below.

Formula 1. $CH_3O_2CCH_2CH_2CHCH_2CHCO_2CH_3$
$CH_3 \quad CH_3$

The mass spectrum* of B indicated fragment peaks typical of dicarboxylic esters (ref. 25). Although the most easily identified peak in a mass spectrum is often the molecular weight peak (M, M=216 for I), under the conditions used no peak for M was observed because of the high probability of bond rupture found with esters. The peak at highest mass to charge ratio obtained was at m/e 185. This peak corresponds to loss of methoxy from the ester group and is normally the highest peak observed in the spectra of these compounds. The presence of fragment peaks at m/e 74 and m/e 88 as well as at m/e 142 (M-74) and m/e 128 (M-88) is further proof of structure I in that these peaks are typical of the rearrangement peaks normally observed in the mass spectra of methyl esters and α-methyl substituted methyl esters. All of the major peaks in the mass spectrum of B would be expected if the compound were dimethyl 2,4-dimethylpimelate.

The specific activity of peaks A and B, on a volumetric basis, was 90% of the specific activity of DMHA.

The infrared spectrum of peak C was remarkably similar to the infrared spectrum of the methyl ester of DMHA with respect to the position of the bands in the region from 5 to 9.5 microns. The spectrum showed that C was a monomethyl ester of an alkyl carboxylic acid. The relative intensities of the bands for C-CH_3 and O-CH_3 were similar to that obtained from the methyl ester of DMHA and indicative of the same type of chain branching. One of the most characteristic bands in the spectrum of peak C was the band in the region of 2.85 microns. This band did not correspond to a carbonyl overtone of the band at 5.76 microns and is at a position where a band for an aliphatic hydroxy compound would be expected (ref. 26). Further proof that peak C is a hydroxy compound is afforded by the fact that the band in the region of 2.85 microns is displaced to higher frequencies upon dilution. Peak C was tentatively assigned the structure of methyl 3-hydroxy-2,4-dimethyl heptanoate formula 2 below and based on the evidence described above.

OH
Formula 2. $CH_3CH_2CH_2CHCHCHCO_2CH_3$
$CH_3 \quad CH_3$

There was not enough material present to obtain a mass spectrum of this compound.

The fraction of ingested activity that appeared in the total lipid from epidydimal fat pad is shown in fig. 5. There is no significant difference between DMHA and propionate at 12 hours. While the amount of activity in the fat pad at 48 hours is slightly higher in animals fed DMHA than in those fed propionate, this difference is small and may or may not be significant.

The residual radioactivity in the lipid-soluble and aqueous-soluble fractions of the carcass are given in tables XI and XII. In addition, the total recovery of radioactivity from all fractions is also given. The activity in the tissues removed for analysis is included in table XI for the

*The mass spectrum was obtained on a CEC21-130 Mass Spectrometer by the Chemistry Department, M.I.T.

purpose of calculating total recovery of the radioactivity administered as propionate. In the case of the experiment with DMHA (table XII), this figure was omitted because it constituted an insignificant fraction of the total.

At 12 hours, the total activity in carcass lipid is greater for DMHA than for propionate, but the values for the two test compounds are similar at 48 hours. At 12 hours in particular, it is not known how much of this lipid-soluble activity is due to unmetabolized DMHA. Although the activity in the nonlipid-soluble fraction of the carcass is greater for propionate than for DMHA at 12 hours, the difference is small. It is questionable whether the difference is significant.

In all but one case (4-12P) the recovery of administered activity is 100±10%. In general, the duplication of measurements reported in this study were within 1 or 2% of the tabulated values. The greatest inherent errors are present in the measurement of activity in nonhomogeneous systems, such as feces, and highly quenched systems, such as occur with saponification of the entire carcass. It is not at all well understood, however, where the major sources of error occur in calculating the recovery of the administered activity.

Discussion

It was the goal of this study to investigate the hypothesis that 2,4-dimethylheptanoic acid (DMHA) would be metabolized by β-oxidation to propionate moieties. This process would yield a net synthesis of glycogen. Therefore, this fatty acid would be, in effect, a nonketogenic source of energy.

The oxidation of the α-methyl carbon of DMHA to CO_2 was less complete and occurred at a slower rate than did the oxidation of carbon 3 of propionic acid. Since the carboxyl groups of even numbered short chain fatty acids are oxidized to CO_2 at essentially the same rate and to the same extent (ref. 27), the oxidation of DMHA apparently is blocked to some extent.

The appearance in urine of a significant amount of the ingested activity of DMHA is further evidence that this compound offered some resistance to oxidation. The sum total of activity in urine and CO_2 is approximately the same at both 12 and 48 hours for both DMHA and propionate. This may be interpreted to mean that the rate of metabolism of both compounds is equivalent.

It is difficult to unambiguously interpret the data obtained in the examination of urine of animals fed DMHA. The bulk of activity in the urine could be accounted for by ω-oxidation of DMHA to 2,4-dimethylpimelic acid (DMPA). The specific activity of DMPA was such that DMHA apparently was its direct precursor. DMPA has not been previously identified as a component of rat urine. Thomas and Weitzel (ref. 28) have shown that dicarboxylic acids are not readily metabolized in the intact animal. The dicarboxylic acids which arise in vivo are generally the product of ω-oxidation of acids of medium chain length (ref. 29), a classification which would certainly include DMHA. Although the fact that any ω-oxidation of DMHA took place is indicative of some hindrance of β-oxidation, the extent of ω-oxidation may be less in a longer chain acid. While Tryding and Westoo (ref. 30) observed a small amount of ω-oxidation of α-methylstearic acid to α-methyladipic and α-methylsuccinic acids, a considerable amount of degradation of the C_{18} chain was requisite to the formation of these dicarboxylic acids. It is not known by what process this degradation occurred. However, the initial step in this process may be assumed to be the oxidation of the terminal methyl carbon atom. Thus, it might be possible to interpret the extensive excretion of the ω-oxidized product of DMHA as a function of the chain length of this acid. Any form of substitution on the main carbon chain may possibly afford some hindrance to β-oxidation.

Further proof of the hindrance to β-oxidation offered by the alkyl substituted structure of DMHA is the tentative identification of the β-hydroxy derivative of DMHA in urine. The process of β-oxidation involves a number of steps and intermediates prior to the shortening of

the carbon chain by 2 carbon atoms. One of these intermediates is the β hydroxy derivative of the original fatty acid. While the normal metabolite β hydroxybutyrate has of course been observed in urine, there is no previous report in the literature of the occurrence of a β hydroxy derivative of a fatty acid in urine. The conversion of β hydroxy DMHA to β keto DMHA may be one of the steps at which alkyl substitution of the main carbon chain sterically hinders attachment of DMHA to the enzyme surface.

The steric hindrance to β oxidation presented by the two methyl groups of DMHA is not nearly as extensive as that found with acids doubly substituted on the α carbon atom. Tryding and Westoo (ref. 30) reported only 3% conversion of the carboxy group of 2,2-dimethylstearic acid to carbon dioxide.

It is not understood whether the ω-oxidation of DMHA is related to its slight toxicity, and therefore, a detoxication reaction. Toxicities of an order of magnitude similar to that of DMHA and its sodium salt have been observed for almost all fatty acids of chain lengths less than 9 carbon atoms (ref. 31), and for triglycerides of these fatty acids (ref. 32). These toxic responses are presumably due to the effect of the low molecular weight fatty acid anions on the central nervous system. Since dietary short chain acids are commonly consumed without evidence of toxic response, the toxicity of DMHA may be expected to be considerably reduced when fed as part of a mixed diet. However, the observed ω oxidation could conceivably be a detoxication mechanism.

Neither DMHA nor propionate was a very effective precursor of fatty acids in the rat. Furthermore, cholesterol and nonsaponifiable material contained little or no activity from these compounds. Acetate, on the other hand, is much more effective than propionate as a precursor of fatty acids in the rat in vivo. This would strongly indicate that DMHA was not metabolized directly through acetate.

The largest amount of activity from DMHA in liver tissue was in glycogen and in the aqueous soluble material. Part of the difference between DMHA and propionate in this respect might be accounted for by unoxidized DMHA. DMHA that had not been metabolized would appear in the fatty acid fraction of the liver while unoxidized propionate would be present in the aqueous soluble fraction. The fact that little or no difference can be found between DMHA and propionate in these fractions at 48 hours would tend to substantiate this interpretation since the animals were refed the test compounds at 24 hours. Furthermore, the overall reduction of lipid soluble activity in the carcass from 12 to 48 hours is greater for DMHA than for propionate.

The classical method of determining whether or not a substance will contribute to a net synthesis of glycogen in vivo has been to feed the substance in question to a fasted animal. Since acetate carbon can be incorporated into glycogen through the citric acid cycle, a high proportion of radioactivity in acetate can be found incorporated in glycogen under conditions where there is little or no net synthesis of glycogen, such as in the fasted animal. The extent of this incorporation is generally small in the fed animal. On the other hand, propionic acid is a direct source of succinic acid and can give rise to a net synthesis of glycogen in the fasted or the fed animal. A comparison of the net incorporation of the 3 carbon of propionate and the α methyl carbon of DMHA into glycogen shows that the contribution to glycogen carbon from each of these compounds is approximately the same. The data would indicate that propionate is an obligatory intermediate in the conversion of DMHA to glycogen, confirming the hypothesis that a multimethylated fatty acid can serve to bypass the difficulties obtained in high fat diets.

SECTION III.

The Direct Small Animal Calorimeter

An extremely sensitive rat size animal calorimeter, capable of automatically recording independent or simultaneous direct and indirect heat output measurements for periods as long as 24 hours has been designed, built, and tested. The direct calorimeter has been thoroughly evaluated and shown to be operational, while the indirect calorimetry system is still in the experimental design state.

The direct calorimeter is based on the thermal gradient principle of Benzinger and Kitzinger (ref. 33) as adapted to the use of multithermocouple heat flow meters by Huebscher et al. (ref. 34). In direct calorimetry, the animal as the heat source is completely enclosed by the heat flow meters. The heat produced by the animal creates a temperature difference between the surface of the heat flow meters facing the animal and the opposite surfaces which are maintained at a constant temperature. The temperature differential or thermal gradient results in a response which is indicative of the rate of heat energy flow through the heat flow meters.

The indirect calorimeter system was designed to measure the changes in oxygen and carbon dioxide content of air passing through the direct calorimeter chamber. Changes in gas concentration are determined by gas chromatography using the technique of Brenner and Ciephinski (ref. 35) and others. Heat production can then be calculated from the caloric value of the oxygen consumed and the carbon dioxide produced.

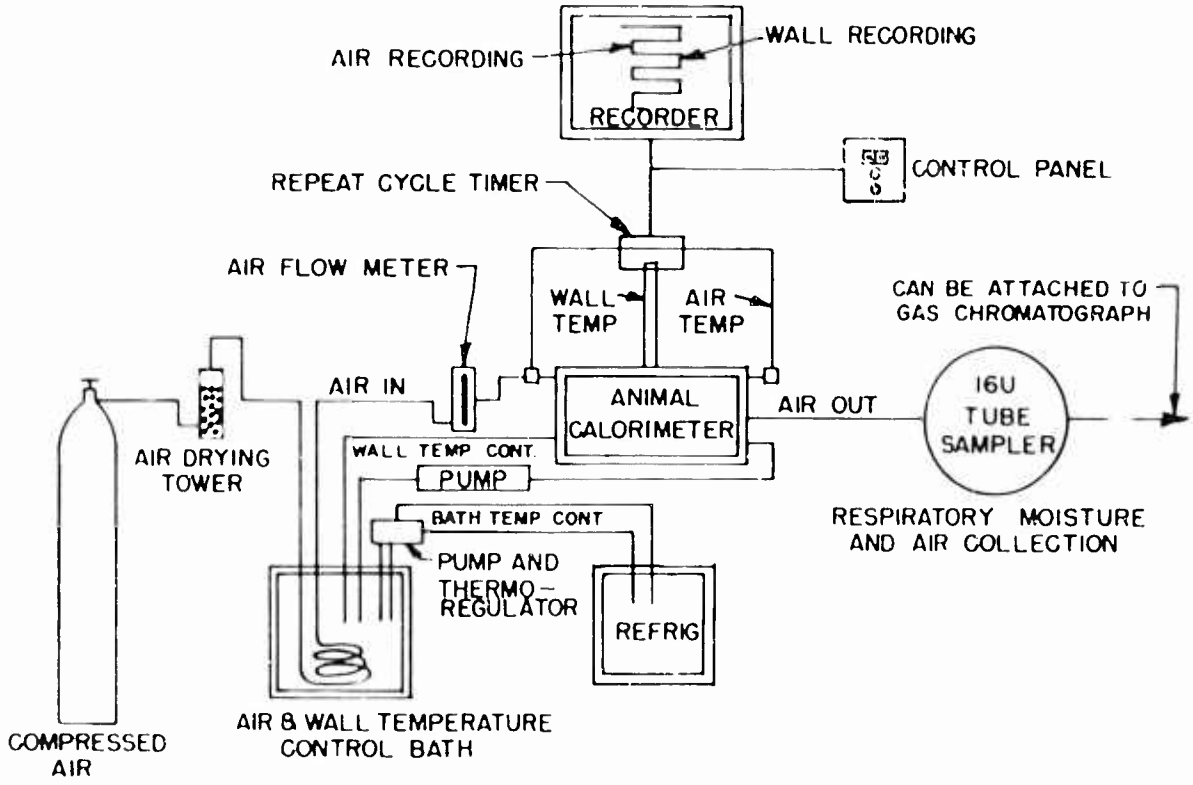


Fig. 7 Flow Diagram of Animal Calorimeter with Attached Equipment

The direct calorimeter has proven to be a reliable and precise instrument. Description of both direct and indirect systems with main construction details are presented but the main emphasis has been directed toward direct calorimetry. A calorimeter flow diagram is given in fig. 7.

For convenience in presentation the description is divided into three sections: (1) the animal cage, (2) the direct calorimeter and (3) the indirect calorimeter.

THE ANIMAL CAGE

A compact metabolism cage which allows the separation and collection of feces and urine was designed for use inside the calorimeter chamber (fig. 8). Complete construction details for this type of animal metabolism cage have been published by Miller et al (ref. 36). The cage is made of ¼-in. mesh stainless steel wire. Separation of feces and urine is accomplished by a triangular screen which allows urine to fall through into a collection pan while the feces roll down the screen into receptacles placed at the sides of the cage. Mineral oil can be added to the urine trough to prevent evaporation.

THE DIRECT CALORIMETER

The main components of the direct calorimeter are: (1) the air intake system which supplies a measurable flow of ventilating air of controlled temperature with provision to measure any sensible heat changes as the air passes through the calorimeter, (2) the sensible heat measuring chamber with supporting equipment, which measures heat loss from the animal by radiation, convection and conduction, and heating respired air, (3) the moisture adsorption equipment to measure energy lost through evaporation of moisture, and (4) the control and recording system. Each of these systems is described below:

Air Supply and Measurement of Sensible Heat Change of Ventilating Air. A constant flow of air is supplied to the animal in the calorimeter from a compressed air tank fitted with a 2-stage reducing valve. Before entering the calorimeter, the air is passed through a combination filter and moisture adsorption trap, a gas flowmeter equipped with a needle valve capable of measuring air intake into the calorimeter in cc/min., and an air temperature control bath.

Constant air temperature is maintained by circulating the air through ¼-in. od copper coils immersed in a constant temperature water bath.

A two-couple copper-constantan thermopile made of No. 30 gauge wire is used to measure any sensible temperature change of the ventilating air passing through the calorimeter. The reference junction is at the inlet port of the chamber and the measuring junction at the ventilating air stream outlet. The imbalance in potential between the two junctions is indicative of the change in temperature of the air stream.

Heat Measuring Chamber. The original calorimeter chamber was constructed of 13 by ⅜ by 13 inch (l by w by h) aluminum alloy wall panels (Alcoa 2024-T4) to give an inside volume of 1 cubic foot. While this size chamber was suitable for direct calorimetry, a smaller chamber was built in order to minimize the response lag for indirect calorimetry. The inside dimension of the latest model chamber is 12 by 7¼ by 10 inches (l by w by h) giving a volume of ½-cubic foot.

As shown in fig. 8, three evenly spaced ⅜-inch entry port holes, which could be fitted with removable plugs, were drilled out of the left side of each of the four wall panels. Similar entry holes were made in two diagonally opposite corners of the top cover. In addition, a ⅛-inch hole was drilled slightly above each center entry hole in the four wall panels to allow for passage of thermocouple wire from the heat flow meters. In the top cover and bottom cover panels the thermocouple wire passage holes were located in the left rear corner. Then, all of the aluminum panels were anodized. This resulted in a 0.001-inch coating of aluminum oxide which furnished necessary electrical insulation between the aluminum panels and the attached heat flow meters.

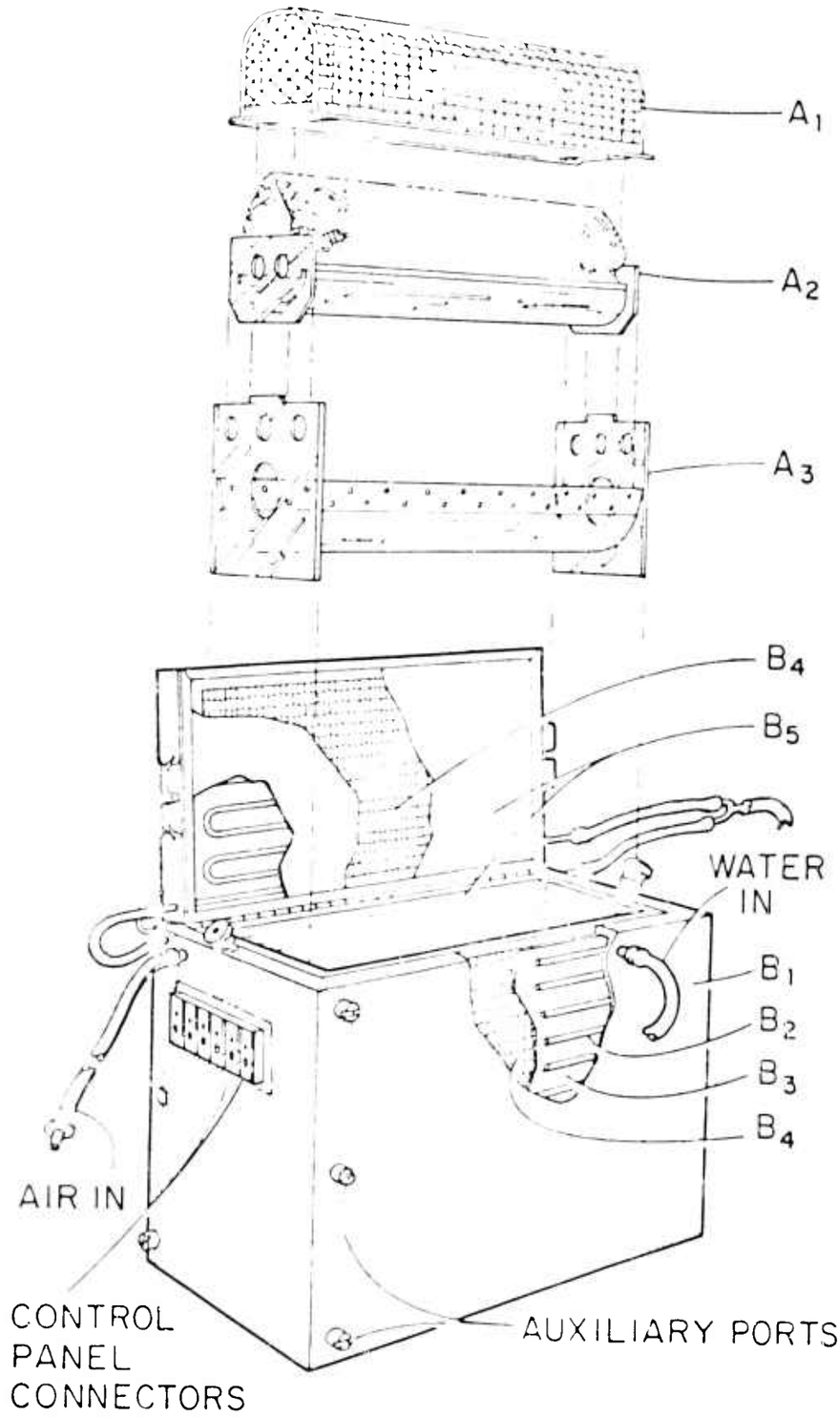


FIG. 8. Animal Cage and Animal Calorimeter Chamber Construction Details

Six heat flow meters were assembled to cover each wall of the calorimeter. The meters consist of notched copper-constantan thermocouple ribbon strips interwoven in a basketweave fashion with glass tape as shown in fig. 9. The strips were placed in parallel and later connected in series to give all copper to constantan junctions on one side of the thermal insulating glass tape layer and all constantan to copper junctions on the other side of the gradient layer.

During assembly, the copper-constantan strips and glass tape were secured at each end to a wooden frame built to enclose an aluminum wall panel. Two inch wide strips of burlap cloth were stapled along the edges of the wooden frame. Then, woven panel size sections of copper constantan ribbon and glass tape were bonded directly to the anodized aluminum panels using an epoxy resin (Scotchcast 3M). After spreading the resin over the surface the meter section and enclosing wooden frame were placed in an air tight plastic envelope which was evacuated with a laboratory vacuum pump. Excess resin and entrapped air were removed from the face of the heat flow meter wall section by gentle pressure and scraping. The burlap cloth along the border absorbed the excess resin. Vacuum was maintained for 6 hours during which time drying was facilitated by heat from a 250 watt infrared light placed above the plastic envelope. After the resin had set in each of the six wall panels, the ends of the copper-constantan ribbon were trimmed and the individual strips connected in series to complete the thermocouple circuitry.

The finished aluminum panels with their attached heat flow meters were assembled to form a cubed chamber. Side and bottom panels were secured together with an epoxy resin (Glyptal) and screws. The top panel which served as a cover was attached by means of hinges and fitted with a sealing neoprene gasket. Knurled nuts were used to fasten down the top cover. Twenty-four gauge thermocouple lead wires from the six individual wall heat flow meters were insulated and brought to the left side of the chamber for later attachment to a junction box. Then, the entire interior, including the faces of the heat flow meters, were painted with one coat of the moisture proof synthetic resin (Glyptal).

In order for one side of the heat flow meter to act as a reference junction, provision was made for the maintenance of a constant temperature at the outer walls of the chamber below that of the inner walls (measuring junction). This was accomplished by covering all six outer walls with $\frac{1}{4}$ inch od copper tubing bent in 1-inch reverse bends. The tubing was soldered to the aluminum walls after being arranged so that the entering water was split into two streams. Each stream cooled two side walls and either the top or bottom of the chamber. Water intake and outlet ports and cover connections are shown in fig. 8.

The copper tubing and walls were covered with an insulating layer of $\frac{3}{4}$ inch asbestos cement. Each individual wall thermocouple lead wire was attached to one of six marked outlets of a thermocouple junction box. The junction box was fitted into the left side of the chamber. Then, the thermocouple lead wires and the walls of the calorimeter were covered with a $\frac{3}{16}$ inch layer of asbestos cement. The final calorimeter covering was a coat of paint.

Air Sampler for Moisture in Ventilating Air. Energy lost by the animal in the form of latent heat of vaporization of moisture is determined by adsorption and gravimetrical measurement. For this purpose, an air sampler was designed to automatically divert the air stream from one to another of 16 adsorption tubes at selected time intervals. The driving motor was geared so that each sampling tube could remain in stream for periods of 15, 30, or 60 minutes. Use of the maximum setting of 60 minutes permits continuous collection of hourly moisture samples over a 16-hour period.

As shown in figs. 10 and 11, which show construction details, the air sampler is essentially a manifold head with 16 outlet ports radiating from a center inlet port. Each outlet port contains a valve which is operated by a continually moving cam of controlled speed. Moisture is adsorbed

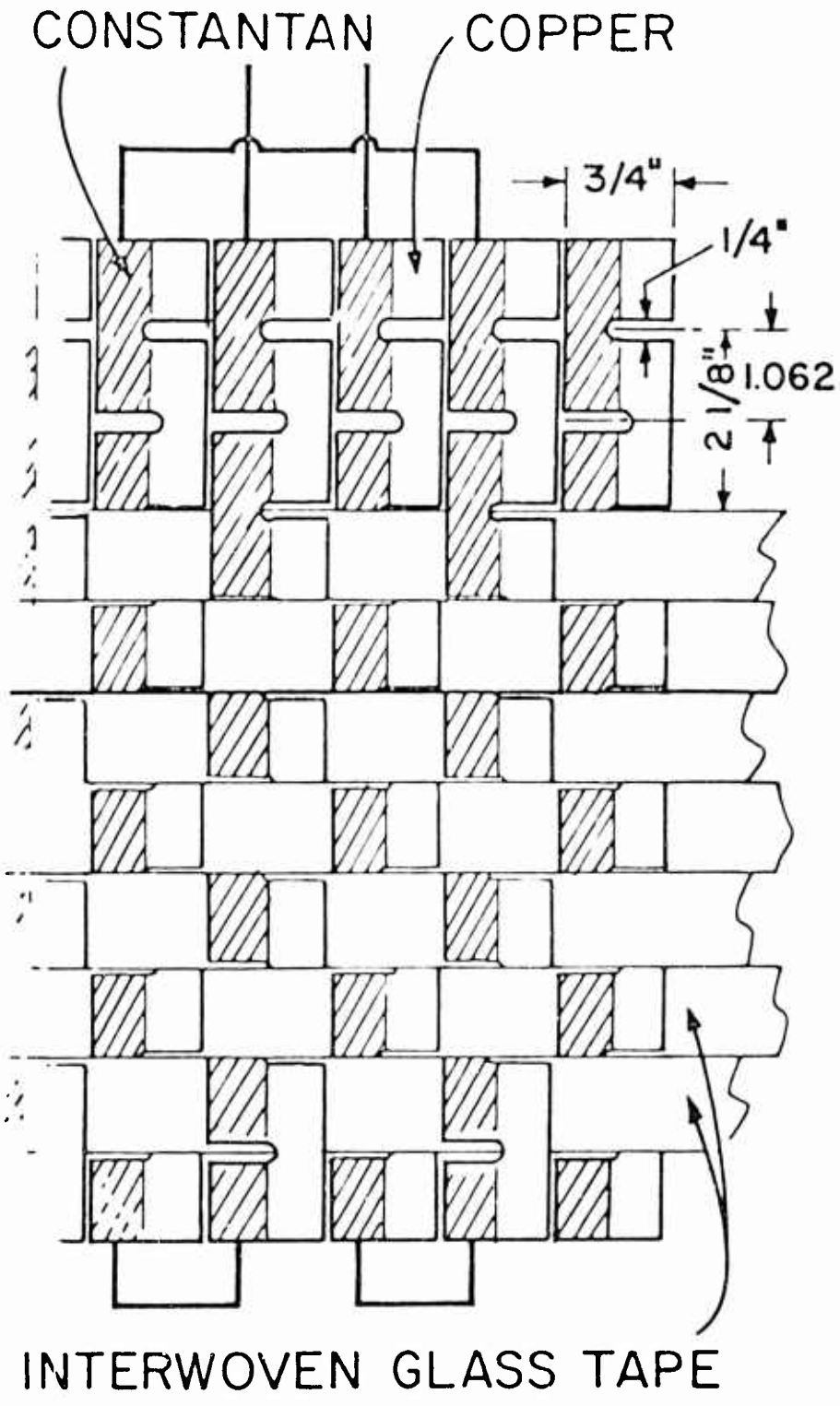


Fig. 9 Construction Details of Notched Copper-Constantan Thermocouple Ribbon Strips Interwoven with Glass Tape

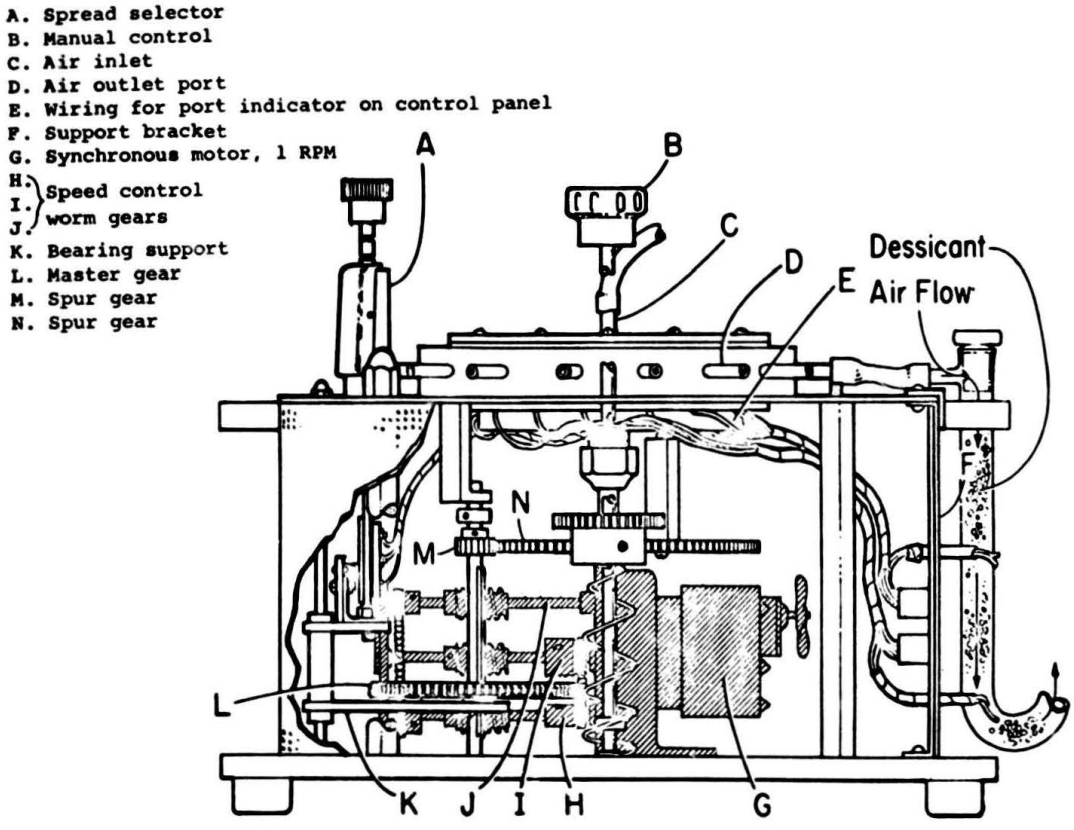


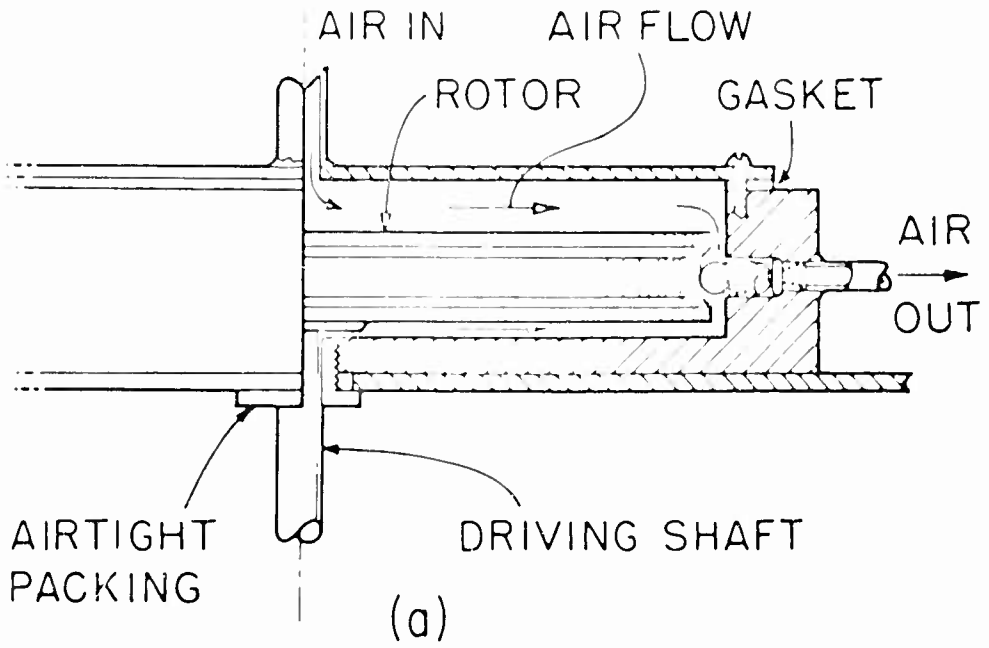
FIG. 10. Schematic Diagram of Respiratory Moisture and Air Sampler

on 8-mesh calcium chloride in 6-inch drying V-tubes fitted with glass stoppers. While it is not necessary for operation, the air sampler was fitted with electric circuitry for identification on the control panel of the adsorption tube in stream.

Control and Recording System. All of the direct calorimeter controls are centered on a single control panel. While it is not necessary to have such an elaborate control panel, the essential features of the panel are switches to operate the experiment timer, constant temperature water bath for the calorimeter outer walls and ventilating air, air sampler for moisture adsorption, and the recorder.

Operation of the calorimeter requires that signals from the heat measuring chamber and from the thermopile measuring temperature change in the ventilating air be constantly monitored. Since both signals could be measured in the 0-1 mv range, only one single channel single pen recorder was required for direct calorimetry measurement. Alternate recording was accomplished through the use of a repeat cycle timer (Bristol Motors RCT-109), a clock motor fitted with a cam, and a snap action switch. The cam was cut so that 2-minute recordings were taken from the heat measuring chamber alternately with 40-second responses from the ventilating air thermopile.

While there is no provision for doing so at present, studies are in progress on suitable means of measuring animal activity and body temperature.



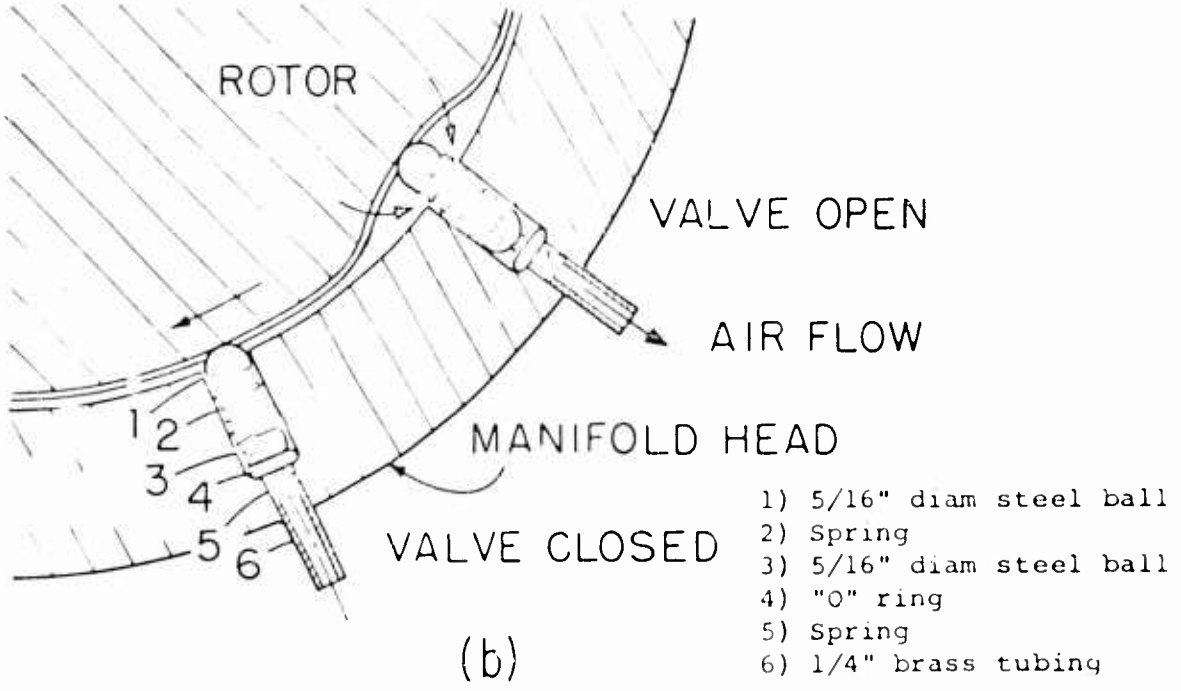


Fig. 11 Schematic Representation of (a) Cutaway Section of Manifold Head and (b) Valves in Open and Closed Positions

THE INDIRECT CALORIMETER

The heat measuring chamber of the calorimeter was calibrated by determining the millivoltage output of the gradient layer heat flow meter units in the presence of a carefully monitored heat source in the form of a 14 ohm nichrome wire heating coil. The energy dissipated in the coil was determined by measuring the voltage drop across the coil and the current supplied to the coil. As represented by the slope of the calibration curve of the heat measuring chamber (fig. 12), the sensitivity of the direct calorimeter is 0.0052 or 0.31 mv/g cal/sec.

As shown in fig. 13, a 50% response occurred in 1.5 minutes and a 100% response in 8.0 minutes. Response times during heating with a constant heat load and the time necessary to return to the baseline after heating was discontinued were similar in that about 8 minutes were required. Numerous tests indicated that the millivoltage response of the heat measuring chamber for a given amount of heat input was constant for a given water bath temperature. Only rapid change in temperature of the surroundings disturbed the equilibrium response.

In actual operation, the test animal, unless in the postabsorptive state (PAS), was intubated with or trained to consume a known amount of diet in a short time. The animal was weighed and put into the cage in the calorimeter chamber. Chamber temperature was maintained at 27°C

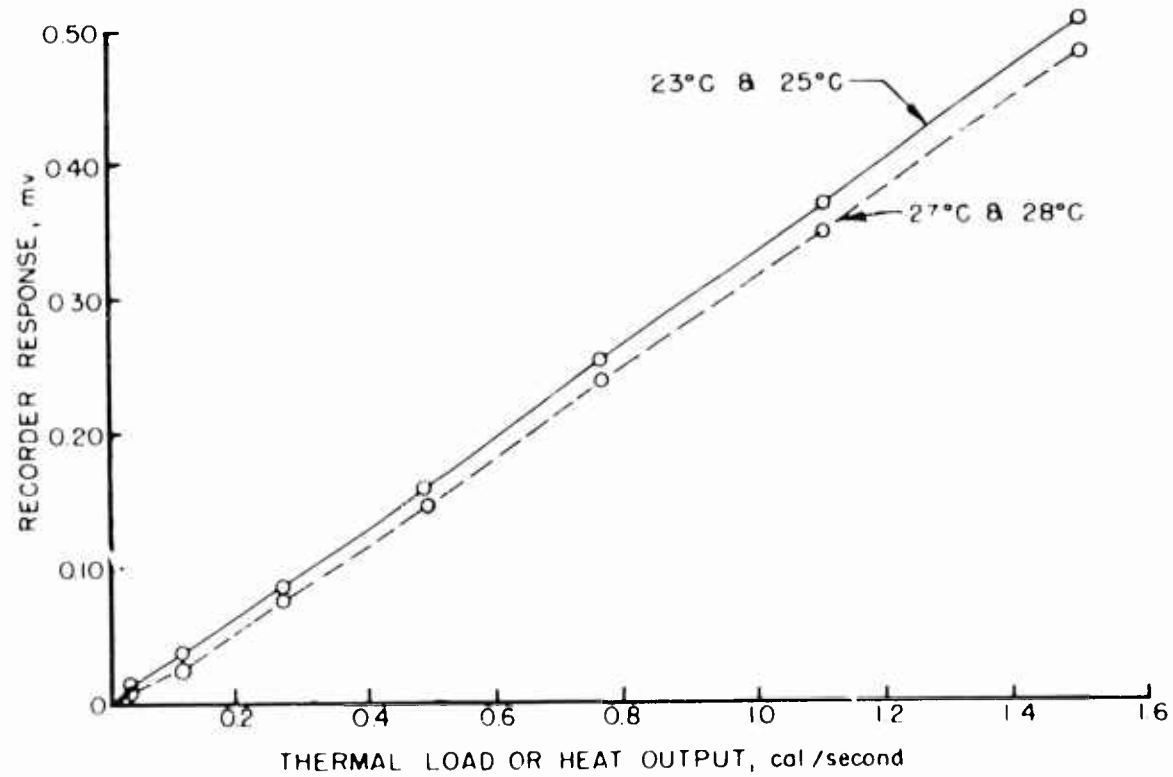


FIG. 12 Calibration Curves of Heat Measuring Chamber, of the Direct Animal Calorimeter

with an average ventilation rate of approximately 1554 cc/min. Then, the moisture adsorption tubes were weighed and inserted into the air sampler. Because of a fast response time, data from the direct calorimeter were recorded as soon as the animal became accustomed to the surroundings in about 15 minutes.

The total heat production of the test animal was calculated as the algebraic sum of the energy represented by (1) the area under the response curve of the heat measuring chamber

(sensible heat), (2) the temperature difference of the ventilating air as indicated by the unbalance of the thermopile placed at the ventilating air inlet and outlet of the chamber (no change detected), and (3) the latent heat of the moisture collected in the adsorption tubes (evaporative heat).

Results and Discussion

The direct calorimeter has given satisfactory performance for periods up to 15 hours during a total operating time of 110 hours. A summary of results from all calorimeter experiments is given in table XIV. In the table, operational runs of more than 1 hour duration are grouped according to the animal used.

Total heat production values for test periods of 4 hours or more varied less when presented on a metabolic body size basis (kcal/$kg^{0.75}$/24 hr) than on a body weight basis (kcal/kg/24 hr). With rat No. 1, the various treatments produced a heat production of 86 to 110 kcal/$kg^{0.75}$/24 hr.

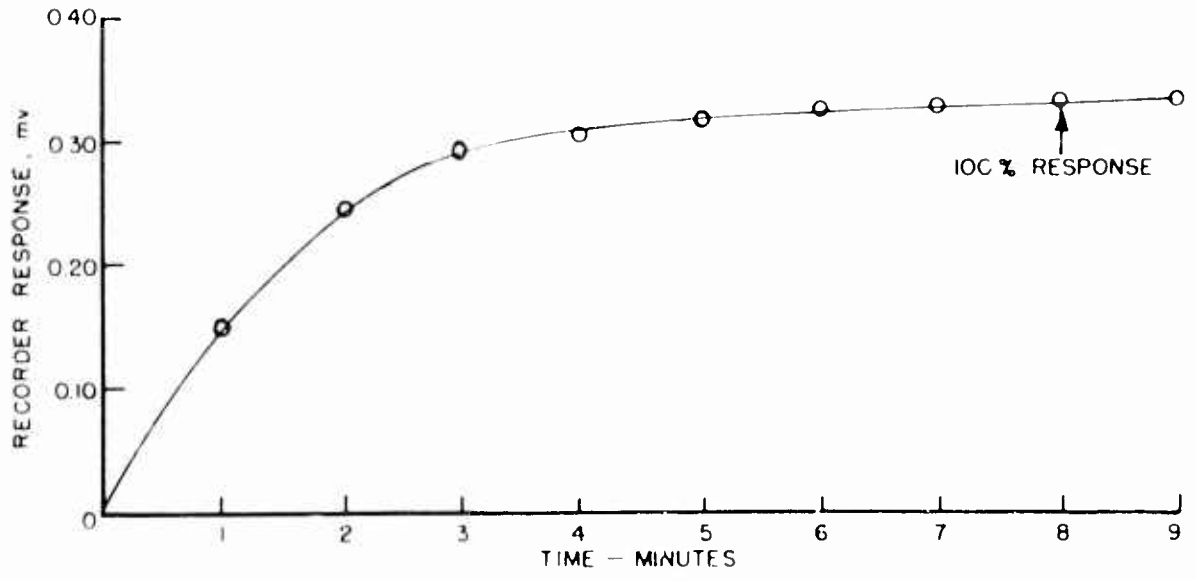


Fig. 13. Time Required to Establish Equilibrium in the Heat Measuring Chamber Under Constant Heat Load

Heat production of rat No. 2 varied from 86 to 116 kcal/$kg^{0.75}$/24 hr. For rat No. 1, about 83% of this heat was in a sensible form, as compared to 86% for rat No. 2.

The data indicate that total heat production on a 24-hour basis was not markedly affected by fasting from 1 to 22 hours (tables XV–XVIII) or by the feeding of a mixed normal type diet just before calorimetry (tables XIX, XX). On a limited number of experiments with intubated dietary energy sources containing 25 Cal, heat production on 1,3-butanediol (tables XXI, XXII) was similar or less than that obtained with water intubation (tables XXIII, XXIV). On the other hand, the response in 2 tests with 25 Cal of intubated sucrose solution (tables XXV, XXVI) was greater than that obtained with water, 1,3-butandiol or feeding a mixed diet. However, in one 9-hour run commencing after feeding a 10% 1,3-butanediol diet (table XXVII), heat production was in the normal range. Thus when 1,3-butanediol is intubated or fed alone, the lower heat production observed may be due to slower absorption of this compound as compared to that of normal food components as sucrose.

Individual test heat production data are given in tables XV to XXVII. These data indicate that, in general, heat production increases slightly during the first and second hour after feeding. This increase does not appear to be significant. In addition, the percentage of the total 24 hour heat production given off for a particular hour appears to be quite constant regardless of the time of feeding.

TABLE XIV

Summary of All Calorimeter Experimental Runs

Rat	Weight Start g	Weight Loss g	Dietary Status	Diet	Hours After Feeding	Run Time hr	Total (kcal/kg$^{0.75}$/24 hr)	Sensible (kcal/kg$^{0.75}$/24 hr)	Evaporative (kcal/kg$^{0.75}$/24 hr)	Total (kcal/kg/24 hr)
6	495	–	PAS*	–––	22	1	107	88	12	128
4	445	–	PAS	–––	22	1	114	84	16	140
5	475	–	PAS	–––	22	1	106	83	17	128
1	335	–	PAS	–––	22	1	117	83	17	154
1	335	–	Fed	Basal†	0	1	108	80	20	142
2	410	–	Fed	Basal	0	1	103	78	22	128
1	357	15	Fed	Basal	5½	15	101	83	17	132
1	374	15	Fed	Basal	5	14	91	82	18	117
1	362	–	Fed	Basal	0	5	87	85	15	113
1	385	–	Fed	Basal	1	11	105	87	13	134
1	367	5	PAS	Water‡	0	4	86	78	22	110
1	367	10	Fed	Surcose§	0	4	110	83	17	142
1	372	12	Fed	BD‖	0	5	86	83	17	110
2	448	14	Fed	Basal	5½	15	103	81	19	126
2	408	12	PAS	–––	0	7	101	88	12	127
2	397	10	Fed	10% BD¶	0	9	104	87	13	131
2	403	10	PAS	Water‡	0	4	101	83	17	127
2	392	8	Fed	Sucrose§	0	5	116	91	9	147
2	408	13	Fed	BD‖	0	6	86	86	14	108

TOTAL 110

*Post absortive state
†20% Casein diet
‡Intubated with 10 ml water
§Intubated with 25 Cal sucrose in 10 ml volume
‖Intubated with 25 Cal 1,3-butanediol in 10 ml volume
¶20% Casein, 10% 1,3-butanediol diet

TABLE XV

Heat Production of Rat Fed 5½ Hours Before Calorimetry

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	1818	6.3	1422	396
2	2337	8.1	1827	510
3	2245	7.8	1827	418
4	2160	7.5	1800	360
5	2019	7.0	1746	273
6	1951	6.8	1665	286
7	1878	6.5	1530	348
8	1783	6.2	1476	307
9	1808	6.3	1557	251
10	1851	6.4	1557	294
11	1678	5.8	1395	283
12	1940	6.7	1665	275
13	1919	6.7	1584	335
14	1739	6.0	1476	263
15	1704	5.9	1503	201
Total	22,830	100.0	24,030	4800

Rat number 1: fasted 16 hours before feeding; 357 g at start.

Water bath temperature: 28°C.

Air flow: 1554 cc/min.

Total heat production: 101 kcal/$kg^{0.75}$/24 hr.
132 kcal/kg/24 hr.

TABLE XVI

Heat Production of Rat Fed 5 Hours Before Calorimetry

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	1655	7	1248	407
2	1729	7	1422	307
3	1754	7	1504	250
4	1746	7	1450	296
5	1867	8	1436	431
6	2086	8	1490	596
7	1795	7	1503	292
8	1776	7	1490	286
9	1826	7	1598	228
10	1728	7	1463	265
11	1751	7	1530	221

TABLE XV (continued)

Heat Production of Rat Fed 5½ Hours Before Calorimetry

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
12	1768	7	1503	265
13	1742	7	1504	238
14	1751	7	1504	247
Total	24,974	100	2645	4329

Rat number 1, 374 g at start, 359 g at finish

Water bath temperature 28° C

Air flow 1554 cc/min

Total heat production 91 kcal/$kg^{0.75}$/24 hr
117 kcal/kg/24 hr

TABLE XVII

Heat Production of Rat Fed 5½ Hours Before Calorimetry

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	2133	6	1584	549
2	2383	7	1773	610
3	2356	7	1908	448
4	2578	8	1989	589
5	2267	7	1800	467
6	2127	6	1719	408
7	2372	7	1827	545
8	2088	6	1584	504
9	2479	7	2097	382
10	2458	7	1935	523
11	2170	6	1827	343
12	2068	6	1827	241
13	2531	7	2178	353
14	2223	7	1935	288
15	2061	6	1746	315
Total	34,294	100	27,729	6565

Rat number 2, 448 g at start, 421 g at finish

Water bath temperature 29° C

Air flow 1554 cc/min

Total heat production 103 kcal/$kg^{0.75}$/24 hr
126 kcal/kg/24 hr

TABLE XVIII

Heat Production of Rat in Postabsorptive State

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	2370	16	2084	286
2	2080	14	1828	252
3	2073	14	1801	272
4	2220	15	1922	298
5	2077	14	1895	182
6	2033	14	1800	233
7	1992	13	1828	164
Total	14,845	100	13,158	1687

Rat number 2: 408 g at start; 396 g at finish.

Water bath temperature: 27°C.

Air flow: 1554 cc/min.

Total heat production: 101 kcal/$kg^{0.75}$/24 hr.
127 kcal/kg/24 hr.

TABLE XIX

Heat Production of Rat Fed 5 Grams Basal Diet Before Calorimetry

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	1509	18	1294	215
2	1870	22	1557	313
3	1687	20	1449	238
4	1594	19	1368	226
5	1737	21	1449	288
Total	8397	100	7117	1280

Rat number 1: fed 5 g 20% casein diet, 362 g at start.

Water bath temperature: 27°C.

Air flow: 1554 cc/min.

Total heat production: 87 kcal/$kg^{0.75}$/24 hr.
113 kcal/kg/24 hr.

TABLE XX

Heat Production of Rat Fed 5.1 Grams Basal Diet Before Calorimetry

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	2342	10	1977	365
2	2233	9	1923	310
3	2066	9	1761	305
4	2024	9	1787	237
5	2073	9	1787	286
6	2040	9	1719	321
7	2155	9	1868	287
8	2011	9	1748	263
9	2043	9	1827	216
10	2179	9	1855	324
11	2100	9	1856	244
Total	23,266	100	20,108	3158

Rat number 1 fed 5.1 g 20% casein diet, 385 g at start, 371 g at finish

Water bath temperature 27 C

Air flow 1554 cc/min

Total heat production 105 kcal/kg$^{0.75}$/24 hr
134 kcal/kg/24 hr

TABLE XXI

Heat Production of Rat Fed 25 Cal BD Before Calorimetry*

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	1811	21	1503	308
2	1600	19	1328	272
3	1564	19	1298	266
4	1698	20	1409	289
5	1730	21	1436	294
Total	8403	100	6974	1429

Rat number 1 intubated with 4.1 g BD and 5.9 ml water (25 cal BD), 372 g at start, 360 g at finish

Water bath temperature 27 C

Air flow 1554 cc/min

Total heat production 86 kcal/kg$^{0.75}$/24 hr
110 kcal/kg/24 hr

*1,3 butanediol

†Commencing with the 2nd hour, the moisture collection equipment failed and the 2 to 5 hour values for evaporative heat are estimates based on previous results with this animal

TABLE XXII

Heat Production of Rat Fed 25 Cal BD Before Calorimetry

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	2192	20	1819	373
2	1828	16	1517	311
3	1828	16	1517	311
4	1778	16	1476	302
5	1746	16	1449	297
6	1812	16	1504	308
Total	11,184	100	9282	1902

Rat number 2, intubated with 4.1 g BD and 5.9 ml water (25 cal BD), 408 g at start, 395 g at finish.

Water bath temperature 27°C.

Air flow 1554 cc/min.

Total heat production 86 kcal/$kg^{0.75}$/24 hr
108 kcal/kg/24 hr.

TABLE XXIII

Heat Production of Rat in Postabsorptive State Intubated with 10 ml Water

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	2078	25	1638	440
2	2111	25	1734	377
3	2105	25	1774	331
4	2148	25	1828	320
Total	8442	100	6974	1468

Rat number 2, 403 g at start, 393 g at finish.

Water bath temperature, 27°C.

Air flow 1554 cc/min.

Total heat production 101 kcal/$kg^{0.75}$/24 hr
127 kcal/kg/24 hr.

TABLE XXIV

Heat Production of Rat in Postabsorptive State Intubated with 10 ml Water

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	1851	28	1544	307
2	1655	25	1149	506
3	1655	25	1375	290
4	1491	22	1143	348
Total	6652	100	5211	1451

Rat number 1: 367 g at start.

Water bath temperature: 27°C.

Air flow: 1554 cc/min.

Total heat production: 86 kcal/kg$^{0.75}$/24 hr.
110 kcal/kg/24 hr.

TABLE XXV

Heat Production of Rat Fed 25 Cal Sucrose Before Caliometry

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	2513	21	2286	227
2	2326	20	2138	188
3	2323	19	2124	199
4	2390	20	2206	184
5	2328	20	2124	204
Total	11,880	100	10,878	1002

Rat number 2: intubated with 6.25 g sucrose in in 10 ml water (25 cal); 392 g at start; 384 g at finish.

Water bath temperature: 27°C.

Air flow: 1554 cc/min.

Total heat production: 116 kcal/kg$^{0.75}$/24 hr.
147 kcal/kg/24 hr.

TABLE XXVI

Heat Production of Rat Fed 25 Cal Sucrose Before Caliometry

Hour	Total Heat* Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative* Heat (cal)
1	1795	21	1490	305
2	2170	25	1801	369
3	2210	26	1827	383
4	2420	28	2016	404
Total	8595	100	7134	1461

Rat number 1: intubated with 625 g sucrose in 10 ml water (25 cal); 367 g at start; 357 g at finish.

Water bath temperature: 27°C.

Air flow: 1554 cc/min.

Total heat production: 110 kcal/$kg^{0.75}$/24 hr.
142 kcal/kg/24 hr.

*Due to mechanical failure, moisture collection apparatus was not functioning during this test period, and the evaporative heat values are estimates based on previous runs with this rat.

TABLE XXVII

Heat Production of Rat Fed 8.2 Grams 10% BD Diet

Hour	Total Heat Production (cal)	Percentage of Total	Sensible Heat (cal)	Evaporative Heat (cal)
1	1996	10	1760	236
2	1751	9	1558	193
3	1910	10	1679	231
4	2071	11	1787	284
5	1990	10	1814	176
6	2575	14	2205	370
7	2328	12	1962	366
8	2331	12	1962	369
9	2304	12	1976	328
Total	19,256	100	16,703	2553

Rat number 2: 397 g at start.

Water bath temperature: 27°C.

Air flow: 1554 cc/min.

Total heat production: 104 kcal/$kg^{0.75}$/24 hr.
131 kcal/kg/24 hr.

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13 ABSTRACT

The aim of these studies was the development of model compounds with which information useful in understanding energy metabolism might be obtained to aid in development of food for space travel. Seven-month studies feeding rats with 1,3-butanediol have been completed. The results of these studies confirm the utilization of this compound as an energy source. Measurement of a number of metabolic parameters at the completion of the study support the contention that 1,3-butanediol is probably metabolized through carbohydrate rather than fat pathways. Metabolism studies with 2,4-dimethylheptanoic acid labeled with C^{14} in the alpha methyl group indicate that this compound as predicted is oxidized through propionate. Design and construction details of the direct animal calorimeter are presented. Results of a limited number of studies with rats fed various diets indicate that the device fulfills its design functions.

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